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PERSISTENCE OF PATHOGENS IN LAGOON-STORED SLUDGE

by

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16. ABSTRACT

The project objective was to investigate pathogen inactivation in lagoon-stored municipal sludges. The in-field lagoons were located in Louisiana (New Orleans) and in Texas (Port Aransas), both semitropical areas of the United States. Each lagoon was filled with 7.56 m^3 of anaerobically digested sludge to which a spike containing a mixture of Salmonella livingstone, poliovirus Type 1, and Ascaris suum eggs was added. The municipal sludge placed in each lagoon was from the respective local area. The field and laboratory data demonstrated that 15 mo of storage was required for pathogen inactivation to meet the U. S. Environmental Protection Agency's (EPA) Process to Further Reduce Pathogens (PFRP) criteria for lagoon-stored sludges in a semitropical climate. In this study, viable Ascaris eggs were inactivated in 15 mo in the New Orleans lagoon where the temperature averaged about 25°C over a 5 mo period. Although a similar temperature was observed for the Texas (Port Aransas) lagoon, all Ascaris eggs were dead after 12 mo of storage, probably because of petroleum organics in the Texas sludge. Salmonella livingstone was inactivated in 4 to 6 mo in both lagoons at a log-reduction rate of 1.2 and 1.6 log Most Probable Number (MPN)/mo/100 mL in New Orleans and Port aransas sediments, respectively. Total coliforms and fecal coliforms declined 2 to 6 logs within 12 mo. Little, if any, die-off of fecal streptococci, either on a volume or a gram dry weight basis, was noted in either lagoon. An increase of total coliforms was observed in both lagoons after 10 mo. Poliovirus Type 1 was inactivated within 12 mo at rates ranging from 0.01 to 0.02 log PFU/mo/100 mL in the sediments of both lagoons.

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FOREWORD

Today's rapidly developing and changing technologies and industrial products and practices frequently carry with them the increased generation of materials that, if improperly dealt with, can threaten both public health and the environment. The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Risk Reduction Engineering Laboratory is responsible for planning, implementing, and managing of research, development, and demonstration programs to provide an authoritative, defensible engineering basis in support of the policies, programs, and regulations of the EPA with respect to drinking water, wastewater, pesticides, toxic substances, solid and hazardous wastes, and Superfund-related activities. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

This research grant has assessed the effectiveness of sludge lagoon storage in a semi-tropical climate for the inactivation of pathogenic microorganisms including bacteria, viruses and parasites. This study demonstrated that sludge lagoon storage in a warm climate can be effective in inactivating these pathogens within 15 months of storage.

E. Timothy Oppelt, Director
Risk Reduction Engineering Laboratory

ABSTRACT

The object of this project was to examine pathogen inactivation in lagoon stored municipal sludges. The lagoons were built in Louisiana (New Orleans) and in Texas (Port Aransas), both in the semi-tropical area of the United States. They were filled with anaerobic digested sludges to which a spike mixture of Salmonella livingstone, Ascaris suum eggs, and Poliovirus Type 1 was added. The field and laboratory data have shown that fifteen months of storage was required for pathogen inactivation to meet Processes to Further Reduce Pathogens (PFRP) criteria in a semi-tropical climate. In this study, viable Ascaris eggs were inactivated in fifteen months in the New Orleans lagoon where the temperature was about 25 to 26° C for about five months while the same range of temperature was observed for the Texas (Port Aransas) lagoon. The Ascaris eggs died after 12 months of storage. This may have been due to the presence of petroleum organics in the Texas sludge. Salmonella livingstone was inactivated in four to six months in both lagoons at a rate of 1.2 and 1.6 log of MPN/month-100 mL, respectively, in New orleans and Port Aransas sediments. Total coliforms and fecal coliforms declined two to six logs within twelve months. The fecal streptococci, either by volume or per gram dry weight, indicated little to no die off in the New Orleans or Port Aransas lagoons. An increase of total coliform was observed in both lagoons after 10 months. Poliovirus was inactivated within 12 months at rates ranging from 0.01 to 0.02 log of PFU/month-100mL in the sediments.

This report was submitted in fullment of Cooperative Agreement Number CR-810289-01-0 by Tulane University under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from August 1, 1982 to July, 31 1986, and work was completed as of July 31, 1986.

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SECTION 1

INTRODUCTION

Lagoon storage of domestic waste sludges as a means of inactivating pathogens has received little attention. Although some observations on the recovery of pathogens from sludge stored in lagoons have been reported, there have been essentially no accompanying control data. In a recent study, it was found that when small aliquots of sludge containing parasite eggs were stored at 25°C, destruction of the *Ascaris* eggs occurred after 10 to 16 months (1). However, when the sludge was stored at 4°C, some *Toxocara canis* and *Ascaris suum* eggs were still viable after 25 months. These results suggested that the storage of sludges in lagoons located in warm climates might be an effective way of inactivating parasite eggs and other pathogens, and in part led to the initiation of this project. If lagoon storage was found to be an effective way of disinfecting sludge the process could be used as a means of treating sludges in tropical regions and in rural regions of the Southern United States (both Western and Eastern) where parasitic infections have a high prevalence.

Sludges from municipal wastewater treatment plants often contain pathogens that are hazardous to humans and domestic animals. Partly because of this, Congress passed the Clean Water Act of 1977 (P.L. 95-217). This act led to the establishment of criteria for the control of pathogens related to the land application of sewage sludge, as set forth in U.S. EPA regulation 40 CFR 257. These criteria specify the minimum level of treatment required for municipal wastewater sludges prior to land application. In addition, EPA designated three categories of municipal sludges destined for agricultural use. They are as follows:

> "Not stabilized" (raw sludge). Not suitable for land disposal; to be incinerated, buried, or heat-dried only.
>
> "Processes to Significantly Reduce Pathogens" (PSRP); e.g., anaerobic or aerobic digestion, heat treatment, lime stabilization, air drying, or composting. Restrictions for land disposal include no grazing for a month on said land, public access control for a period of one year, and an 18 month wait before crops for direct human consumption may be grown.
>
> "Processes to Further Reduce Pathogens" (PFRP); no disease related restrictions on the land use of the sludge. The PFRP includes such high energy processes as irradiation, thermal processes (pasteurization, heat treatment, thermophilic digestion) and certain types of composting.

The PSRP process, as noted by EPA (2), may be defined as a process that reduces pathogenic viruses by one log or 90 percent, or indicator bacteria (fecal and total coliforms) by two logs or 99 percent. These currently listed processes are shown in Table 1. The criterion for a PFRP process is the reduction of pathogens to below detectable limits; i.e., one Plaque Forming Unit (PFU) for viruses, three MPN for Salmonella and one viable helminth egg per 100 mL of sludge. A listing of PFRP is presented in Table 2. As of November, 1986, an EPA task force was established to review, on a case by case basis, processes not listed in Tables 1 and 2, but which attain the required pathogen reductions (3).

In the last three years, the disposal of municipal sludges in some eastern states (New Jersey, Maine, and Massachusetts) has been changing, due to legislative mandates. For example, New Jersey has ruled that after March 15, 1985, municipal sludges can no longer be disposed of in a

TABLE 1. REGULATORY DEFINITION OF PROCESSES TO SIGNIFICANTLY REDUCE PATHOGENS (PSRP) (3)

- Aerobic Digestion: The process is conducted by agitating sludge with air or oxygen to maintain aerobic conditions at residence times ranging from 60 days at 15°C (59°F) to 40 days at 20°C (68°F), with a volatile solids reduction of at least 38 percent.
- Air Drying: Liquid sludge is allowed to drain and/or dry on underdrained sand beds, or on paved or unpaved basins in which the sludge depth is a maximum of 9 inches. A minimum of 3 months is needed, 2 months of which temperatures on a daily basis must average above 0°C (32°F).
- Anaerobic Digestion: The process is conducted in the absence of air at residence times ranging from 60 days at 20°C (68°F) to 15 days at 35°C (95°F) to 55°C (131°F), with a volatile solids reduction of at least 38 percent.
- Composting: Using the in-vessel, static aerated pile, or windrow composting methods, the solid waste is maintained at minimum operating conditions of 40°C (104°F) for 5 days. For four hours during this period, the temperature must exceed 55°C (131°F).
- Lime Stabilization: Sufficient lime is added to reach and maintain a pH of 12 for two hours.
- Other Methods: Other methods or operating conditions may be acceptable if pathogens and the vector attraction of the wastes (volatile solids) are reduced to an extent equivalent to the reduction achieved by any of the above methods.

TABLE 2. REGULATORY DEFINITION OF PROCESSES TO FURTHER REDUCE PATHOGENS (PFRP) (3)

- Composting: Using the in-vessel composting method, the solid waste is maintained at operating conditions of 55°C (131°F) or greater for 3 days. Using the static aerated pile composting method, the solid waste is maintained at operating conditions of 55°C (131°F) or greater for 3 days. Using the windrow composting method, the solid waste attains a temperature of 55°C (131°F) or greater for at least 15 days during the composting period. Also, during the high temperature period, there must be a minimum of 5 turnings of the windrow.

- Heat Drying: Dewatered sludge cake is dried by direct or indirect contact with hot gases; the moisture content is reduced to 10 percent or lower. Sludge particles reach temperatures well in excess of 80°C (176°F), or the wet bulb temperature of the gas stream in contact with the sludge at the point where it leaves the dryer is in excess of 80°C (176°F).

- Heat Treatment: Liquid sludge is heated to temperature of 180°C for 30 minutes.

- Thermophilic Aerobic Digestion: Liquid sludge is agitated with air or oxygen to maintain aerobic conditions at residence times of 10 days at 55°C (131°F) to 60°C (140°F), with a volatile solids reduction of at least 38 percent.

- Other Methods: Other methods of operating conditions may be acceptable if pathogens and the vector attraction of the wastes (volatile solids) are reduced to an extent equivalent to the reduction achieved by any of the above methods.

Any of the processes listed below, if added to a PSRP, will further reduce pathogens.

- Beta Ray Irradiation: Sludge is irradiated with beta rays from an accelerator at dosages of at least 1.0 megarad at room temperature, approximately 20°C (60°F).

- Gamma Ray Irradiation: Sludge is irradiated with gamma rays from certain isotopes, such as 60 cobalt and 137 cesium at dosages of at least 1.0 megarad at room temperature, approximately 20°C (60°F).

- Pasteurization: Sludge is maintained for at least 30 minutes at a minimum temperature of 70°C (158°F).

TABLE 2 (continued)

- Other Methods: Other methods or operating conditions may be acceptable if pathogens are reduced to an extent equivalent to the reduction achieved by any of the above add-on methods.

landfill (4). Incinerating, except in northern New Jersey, has been ruled out because emission levels are currently at their limits. New Jersey's Department of Environmental Protection is confronted with the fact that municipal sludges must be reused or shipped out of state. Because only 50 tons of municipal sludge can be applied to an acre of farm land over the total life span of the land, it is necessary that municipal sludge treatment processes be developed so that sludge will be suitable for reuse such as fuel, soil amender, food source, liming agent, pozzolanic product, autoclaved product, gypsum product, etc. The reuse potential of sludge can be ascertained by determining its characteristics. However, all sludges reused in New Jersey must be treated to achieve PFRP status.

As a result of the passage of 1987 Clean Water Act, regulations will be expanded by the Fall of 1989. In the past, regulations were directed to the process, rather than to the product. As a result of States going to a product related end-use of sludge, the regulations will be based on a product quality, rather than process performance. Regrowth problems were not addressed by the previous regulations.

PATHOGENS OF CONCERN

Because of the potential health problems resulting from the re-use of human wastes, there has risen a concern about pathogen survival. Initial levels of microbial pathogens present in sludges were noted by Pedersen (5) (Table 3). Even though mesophilic anaerobic digestion reduces pathogen concentrations from one to two orders of magnitude, residual levels for total coliforms, fecal coliforms and fecal streptococci remain in the range of 10^5 to 10^8 organisms per gram dry weight after sludge treatment (6).

Pathogens can produce varied responses in individuals exposed at relatively low concentrations (Table 4). To insure safety of the environment and human health, the further reduction of the very low levels of pathogenic bacteria is necessary prior to the release of these waste sludges.

Although the number of parasite species found in sewage sludges in the United States may exceed 30 (7), the number likely to be transmitted through sludges to man is much less. Parasites that would seem to have the

TABLE 3. DENSITY OF PATHOGENS IN RAW SLUDGES: GEOMETRIC MEAN OF ORGANISMS PER GRAM DRY WEIGHT (5)

Organism	Primary	Secondary	Mixed
Total coliforms	1×10^8	7×10^8	1×10^9
Fecal coliforms	2×10^7	8×10^6	2×10^5
Fecal streptococci	9×10^5	2×10^6	4×10^6
Salmonella spp.	4×10^2	9×10^2	3×10^2
Pseudomonas aeruginosa	3×10^3	1×10^4	3×10^3
Bacteriophage	1×10^5 PFU	---[a]	---
Enteric Virus	2×10^3 PFU	---	5.0×10^2

---

[a] Not done

TABLE 4. INFECTIVE DOSE OF ENTERIC BACTERIA (6)

Bacterium	No Infection	Percent of Volunteers Infected			
		1-25	26-50	51-75	76-100
Enteropathogenic Escherichia coli	10^4	10^6	10^8	10^8-10^{10}	10^{10}
Salmonella typhi	10^3	10^5	10^5-10^8	---[a]	10^8-10^9
Salmonella spp.	10^4-10^6	10^6	10^7	10^7-10^8	
Shigella	---	10^1-10^2	10^2-10^4	10^3	10^4
Vibrio cholerae					
Buffered	10^1	---	10^3-10^8	10^4-10^6	---
Unbuffered	10^4-10^{10}	---	10^8-10^{11}	---	---

---

[a] Not done

greatest potential for transmission through domestic sludges to man, and in some cases animals, are listed in Table 5. Each of the parasites listed has a resistant stage that may survive the wastewater treatment process. For the first seven parasites, the resistant stage is an egg that must undergo development in the soil before it becomes infective for the definitive host or man. The minimum time required for this development varies approximately two to four weeks and depends on the presence of favorable environmental conditions (sufficient moisture and oxygen, and optimum temperature). The eggs of *Taenia saginata, Taenia solium* and *Echinococcus granulosus* are already infective when passed in the feces of the host and are likely to still be infective when they enter sewage. The oocysts of *Toxoplasma gondii* are not infective when passed in the feces of cats but can develop to the infective stage in three to four days under suitable environmental conditions (7).

TABLE 5. PARASITES HAVING THE GREATEST POTENTIAL FOR TRANSMISSION TO MAN THROUGH DOMESTIC SLUDGES IN THE UNITED STATES (7)

Parasite	Stage	Definitive Host	Location of Infect. Stage[a]
Ascaris lumbricoides	Egg	Man	"Soil"
Ascaris suum	Egg	Pig	"Soil"
Toxocara canis	Egg	Dog	"Soil"
Toxocara cati[b]	Egg	Cat	"Soil"
Trichuris trichiura	Egg	Man	"Soil"
Trichuris suis[b]	Egg	Pig	"Soil"
Trichuris vulpis[b]	Egg	Dog	"Soil"
Taenia saginata	Egg	Man	Cow
Taenia solium	Egg	Man	Pig (Man)
Echinococcus granulosus	Egg	Dog	Sheep, pig, etc. (Man)
Toxoplasma gondii	Oocysts	Cat	"Soil", Mammals, (Man)

[a] Where stage infective for definitive host develops, i.e., free in environment (soil), or in intermediate host (cow, etc.).

[b] Medical importance questionable.

Of the human parasites on this list, the eggs of *Ascaris* spp. are the ones that are most commonly found in raw and stabilized sludges in this country (7-11), and are probably the most important. *Ascaris* eggs are resistant to a wide range of chemical and physical conditions and are generally considered to be the most resistant form of any parasite. Ascaris eggs have been reported to persist in the soil for over five years (12,13), and are capable of surviving longer in sewage sludges and in soil than are most bacteria, viruses and fungi (14). For this reason, Ascaris was selected as a parasite indicator organism for monitoring the effectiveness of sludge treatment processes or disinfection procedures. Although the eggs of other helminths are less resistant than those of Ascaris, they are nevertheless capable of surviving for months or years under favorable conditions. The oocysts of the protozoan Toxoplasma gondii are also quite resistant. They have been reported to survive for at least 18 months in the soil under natural conditions (15).

EFFECTIVENESS OF EXISTING TECHNOLOGY

The information in Tables 6 and 7 was originally developed by Stern and Farrell (16), but has been expanded to include more recent work regarding the relative effectiveness of processes that are generally considered suitable for disinfecting sludges. While general methods such as pasteurization, heat treatment, and heavy chlorination appear to be excellent for the destruction of certain pathogens, they may be undesirable due to associated costs or to resultant changes in sludge properties and/or separated liquid characteristics.

In Table 8 it is shown that conventional sludge treatment processes may not be totally effective in removing or destroying parasites. Conventional domestic sludge treatment processes can be divided into two categories: 1) stabilization processes (decreasing bulk organics, odor, and pathogen content of sludges); and 2) inactivation processes (making the handling and disposal of sludges safer and more economical). It has been found that these two major categories affect parasites in different ways. In sludge stabilization processes, aerobic or anaerobic environments are produced which may or may not raise the temperature to lethal levels. Because most parasite eggs require an oxygen level for development above that in the host's gut, anaerobic digesters tend to inhibit while aerobic digesters tend to accelerate their development. As expected, these processes will kill eggs if either the anaerobic or aerobic processes are carried out at temperatures ($>55^{o}C$) which are lethal to parasites. Some sludge dewatering and disinfection processes will destroy the eggs by increasing the temperature, as in incineration and composting, or by greatly reducing the moisture content, as in drying beds. In more exotic cases, eggs may be destroyed by disruption by sonication, radiation, or microwaves (7).

Reimers et al. (7) found that conventional sludge stabilization treatment processes (e.g., mesophilic anaerobic or aerobic digestion) were not very effective in destroying parasite eggs. The number of viable Ascaris and Toxocara eggs per unit dry weight of sludge actually increased during these processes due to the loss of the dry mass of sludge solids that occurred in the digestion processes. On the other hand, the use of drying beds was found to be consistently effective in destroying parasites.

TABLE 6. EFFECTIVENESS OF STANDARD SLUDGE DISINFECTION PROCESSES (5, 16-19)

	Removal or Inactivation		Removal or Inactivation		
Disinfection process	Indicator[a] organisms	Pathogenic bacteria (Salmonella)	Viruses	Parasites (Ascaris lumbricoides)	Regrowth problems
Standard processes					
Lagoon storage (pilot)	E[b]	E	E	E	–[c]
Drying beds (moisture content temp. control)	G[d]	E	G+	E	x[e]
Long term anaerobic storage (6 mo.)					
Laboratory batch test at 4°C	F+[f]	E	P[g]	P	–
Laboratory batch test at 20°C	E	E	E	P	–
Temperature (heat) – time processes					
Anaerobic digestion (35°C)	F	F	P+	P	–
Anaerobic digestion (52°C)	G	E	G	P	–
Aerobic digestion	F	P+	P	P	–
Anaerobic digestion (60°C)	G	E	E (Est.)[h]	E (Est.)	–
Composting (>60°C)	G+	E	G+	E	x
Pasteurization (70°C, 1/2 to 1 hr)	G	E	E	E	x
Pasteurization (70°C, 1 to 2 hr)	E	E	E	E	x
Heat Treatment (195°C)	E	E	E	E	x
Heat Drying	E	E	E	E	x

[a] Indicator organisms: Total coliform, Fecal coliform, Fecal streptococci

[b] Excellent, below detectable levels

[c] Inhibits growth

[d] Good, more than 3 logs reduction

[e] Regrowth can be a significant problem

[f] Fair, 1-3 logs reduction

[g] Poor, less than 1 log reduction

[h] Estimated

TABLE 7. EFFECTIVENESS OF INNOVATIVE SLUDGE DISINFECTION PROCESSES (5, 16-19)

Disinfection process	Removal or Inactivation		Removal or Inactivation		
	Indicator[a] organisms	Pathogenic bacteria (Salmonella)	Viruses	Parasites (Ascaris lumbricoides)	Regrowth problems
Chemical treatment					
Lime treatment (pH>12)	E[b]	E	E (Est.)	P[c]	x[d]
Heavy chlorination (C12 1500 mg/L)	E (Est.)	E (Est.)	E (Est.)	P (Est.)	x (Est.)
Ammonization (1%)	E	E	E	E	x
Applied fields					
Ultrasound	P to E	P to E	P to E	P	x
Gamma Irradiation (300-400 krad)	G+[e]	E	F	E	x
Gamma Irradiation (1000 krad)	E	E	E	E	x
Gamma Irradiation (300-4000 krad, 55°C)	E	E	E	E	x
High energy electron irradiation (1000 krad)	E	E	G+	E	x

[a] Indicator organisms: Total coliform, Fecal coliform, Fecal streptococci

[b] Excellent, below detectable levels

[c] Poor, less than 1 log reduction

[d] Regrowth can be a significant problem

[e] Good, more than 3 logs reduction

TABLE 8. THE INFLUENCE OF SLUDGE TREATMENT PROCESSES ON PARASITE EGGS

Unit Operation	Effectiveness	Sources
	Stabilization process	
Anaerobic digesters	(Retards eggs development, Increases destruction with increased temperature)	7,14,25
Aerobic digesters	Promotes egg development (increases destruction with inincreased temperature)	18
	Decontamination processes	
Incineration	100% destruction	18
Drying beds	100% kill at <5% moisture content Moisture content may vary with temperature	26
Composting	100% effective if all matter reaches 60°C for at least 2 hrs	18,27
Routine chlorination	No effect	28,29,30
Sonication	80% effective at 30-50KHz and 600 Watts (80%)	7,18,21,31
Gamma radiation	100% effective at 200 KRADs	18,32,33
Heat	100% effective about 70°C and 30 minutes or in less time at higher temperatures. Effectiveness depends on temperature and exposure time but temperatures below 45°C appear to have no effect	14,18
Lagoon storage	50-100% (depends on time and temp.)	18
Lime stabilization	80-100% (depends on digestion, time, and temperature)	18
Ammonification	Up to 95% (depends on dosage and pH)	18

A log-log correlation was found between the density of viable parasites and the moisture content in drying bed sludges. The inactivation of parasite eggs increased with the decrease of moisture content of the drying bed sludges. The densities of viable Ascaris and Toxocara eggs in the drying bed sludges, as related to the sludge moisture content, were analyzed for each season. The lowest moisture level at which all Ascaris or Toxocara eggs were inactivated was 5% in the fall, 7% in the winter, 8% in the spring, and 15% in the summer. Evidently, both temperature and reduction in moisture content played a part in the inactivation of these parasites.

Recently, the effective destruction of pathogens by anaerobic digestion has been shown to be fallacious (20-23). Even though most microorganisms are thought to be destroyed during anaerobic sludge digestion, a number of microbes survive this process. These include bacteria (Salmonella typhi), parasites (various helminth eggs), and viruses (poliovirus) (20-24). Survival in the aforementioned categories has caused many health authorities to remain skeptical about the practice of land disposal of waste sludges, since once a sludge is applied to the land, viable pathogens remaining in the waste may become dispersed in the environment. Many pathogens are known to survive in soil for months or years, such as the tubercle bacilli and eggs of the nematode Ascaris.

As noted by Stern and Farrell (16), the effectiveness of the sludge disinfection processes depends on each sludge particle and associated liquid receiving adequate treatment. Also to be noted is that a count below a detectable level does not guarantee complete absence of a specific pathogen. For example, below a detectable level for an indicator organism may mean that the density is less than 3 per 100 mL of sludge; for pathogenic bacteria, the density is less than 3 per 100 mL of sludge; and for viruses and parasites, the organisms are not detectable using the best isolation methods currently available. The more intense the disinfection treatment (e.g., high temperature treatments or high levels of irradiation) the less the chance of survival of pathogenic organisms.

SLUDGE LAGOON STORAGE

Only a few observations have been made concerning the presence and survival of helminth eggs in lagoon-stored sludge in this country. Arther (34) examined sludge from lagoons in the Chicago Sanitary District in Fulton County, Illinois. On four occasions from May, 1976 to October, 1977, he recovered an average of 203 Ascaris, 173 Toxocara, 48 Toxascaris leonina and 36 Trichuris eggs per 100g dry weight. When these eggs were cultured, the percentage of eggs developing to the infective stage was 24 percent for Ascaris, 10 percent for Toxocara, 43 percent for Toxascaris leonina, and 6 percent for Trichuris. Unfortunately, no information was provided on the length of time that the sludge had been stored in a lagoon.

In the study of Reimers et al. (7), only one sample of lagoon-stored sludge was collected. Nevertheless, from one plant in central Mississippi, the following number of eggs were recovered from 100 ml (viability based on morphologic criteria): Ascaris, 1730 (13% viable); Trichuris trichiura, 21

(0% viable); T.vulpis, 192 (78% viable); and Toxocara, 230 (0% viable). It is not known how long the eggs had been in storage, but the lagoons were designed to store sludge for three years.

Although little information is available on the survival of helminth eggs in lagoon-stored sludge, the results of laboratory experiments in which helminth eggs were stored in digested sludges at 4°C and 25°C were recently reported (1). Complete inactivation of Ascaris suum eggs was observed in both aerobically and anaerobically digested sludges after 16 months storage at 25°C. Under similar conditions, Toxocara canis and Trichuris vulpis eggs were completely inactivated much sooner. However, when stored at 4°C, A. suum eggs were still viable after two years. The die-off of selected bacteria and viruses, including some pathogens, has been found to occur at a greater rate than for the eggs of Ascaris under the same conditions (35). At temperatures of 20°C or greater, the minimum time required to obtain a reduction of one log was found to be about one month for bacteria and two months for viruses. At temperatures less than 20°C, the storage time required to achieve the same level of reduction was more than six months for bacteria and more than eight months for viruses.

Pedersen (5) reported a three log reduction of pathogenic bacteria in lagoon-stored anaerobically digested sludges within four months at 20°C; but at 4°C there was no reduction of fecal streptococci. In an earlier study by Stern and Farrell (16), Salmonella sp. behaved similarly to indicator bacteria. At 20°C, a greater than 1-log reduction of Salmonella sp. was observed in less than one month, and at 4°C, a 1-log reduction was not observed until after six months. Thus, reduction appears to be a function of such variables as temperature, sludge composition, storage time, and type of sludge stabilization.

Lagoon storage of sludge appears to be very capable of inactivating viruses by one to three logs within six months to two years. Viruses, like bacteria, appear to be affected by storage time and temperature. Stern and Farrell (16) noted that indigenous enteroviruses had a 0.5 log reduction after six months of 4°C, while a greater than 1 log reduction occurred within 2 months at 20°C. Sattar and Westwood (36) conducted a 15 months field study on the lagoon storage of anaerobically digested sludges and found a complete inactivation of viruses between 8 - 13 months. Bitton et al. (37), in a field study on the lagoon storage of combined aerobic and anaerobic digested sludges in Florida (a warm semi-tropical climate), observed complete inactivation of detectable viruses within two months. In a study by Farrah et al. (38), the influence of lagoon storage was investigated for virus inactivation, and they observed that the enterovirus inactivation appeared to be similar to fecal coliform inactivation. Interestingly, in Farrah's study, polioviruses were reported to make up 90% of all the viruses observed in the initial part of the study, but in the latter stages, the polioviruses made up only 70% of the enteroviruses, while the echoviruses and Coxsackie viruses had become the most common viruses identified. In summary, the inactivation of viruses appears to be a function of temperature, storage time, sludge digestion and sludge composition.

SECTION 2

CONCLUSIONS

Ascaris suum eggs were inactivated within fifteen months of storage in the New Orleans, Louisiana and Port Aransas, Texas lagoons. Both localities are in semitropical zones. In the Texas lagoon, *Ascaris* inactivation was at a faster rate and this may have been due to the petroleum contaminants in the sludge. *Salmonella livingstone* and Poliovirus type I were inactivated within six months of storage. The indicator bacteria dropped two to six logs for total and fecal coliforms but the fecal streptococci decreased very little.

For the New Orleans municipal sludge, changes in abiotic parameters that correlated with *Ascaris* egg inactivation included only volatile solids. Fecal streptococci inactivation correlated with the fluctuation of volatile solids and temperature. The inactivation of other indicator organisms and *Salmonella* was not observed to correlate with any abiotic changes. For the Port Aransas municipal sludge, no abiotic parameters were correlated with *Ascaris* egg inactivation. Poliovirus inactivation correlated with changes in pH, volatile solids and total solids. Fecal streptococci inactivation was correlated only with conductivity. The abiotic variables could not be used as indicators of inactivation but these abiotic parameters were related to the mechanism by which the die-off occurred. With the New Orleans municipal sludge, die-off of pathogens appeared to be a result of temperature, while *Ascaris* egg die-off in the Texas petroleum contaminated sludge was related more to petroleum residues which were estimated to be around fifteen to twenty percent by volume.

Finally, the die-off data for both lagoon sites indicated pathogen reductions within fifteen to eighteen months, and was in accordance with published processes to further reduce pathogens (PFRP) in sludge, treatment processes, or process schemes.

SECTION 3

RECOMMENDATIONS

Results of this study indicate that lagoon storage of municipal sludges under certain conditions in semi-tropical climates can be used to inactivate pathogens. It was also observed that Ascaris eggs are far more resistant to inactivation than bacteria and viruses following storage of municipal sludges in lagoons under semi-tropical conditions. Based on these findings the following is recommended:

1) Drying bed studies should be initiated using raw, aerobically, and anaerobically treated sludges so that exact stabilization conditions can be determined for producing sludges meeting PFRP criteria.

2) The applicability of using combined treatment processes (digestion followed by lagoon and/or drying bed storage) should be investigated as feasible and simple approaches for the inactivation of enteropathogens in sludges being processed in rural and/or small Publicly Owned Treatment Works (POTW).

3) Efforts should be made to determine the effects of petroleum hydrocarbons on the inactivation of enteropathogens in non-hazardous petroleum sludges, petroleum contaminated municipal sludges, and pit muds being co-disposed with municipal sludges.

4) Another study is needed to determine the appropriate controls for studies on the survival of Ascaris eggs in different sludges or sludge products.

SECTION 4

OBJECTIVES

The overall objective of this study was to evaluate the effectiveness of sludge storage in lagoons as a method of inactivating pathogens. A specific objective was to determine whether the storage of selected anaerobic sludges in lagoons in areas where the mean ambient temperatures approach 25°C could be an effective way of inactivating selected parasite eggs (*Ascaris suum*), *Salmonella*, fecal coliforms, fecal streptococci, and enteroviruses (Poliovirus Type 1). An additional objective was to detect changes in sludge characteristics which could be correlated with pathogen inactivation. Finally, the effectiveness of the sludge lagoon storage was to be assessed as a potential PFRP component to conventional sludge treatment process schemes (i.e., anaerobic digestion and sludge lagoon storage) leading to reuse potentials.

SECTION 5

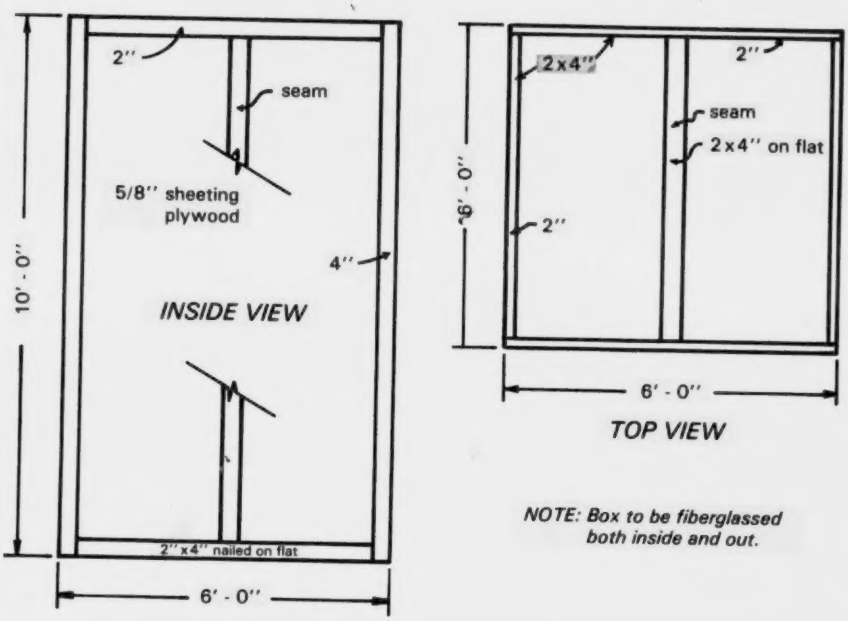
MATERIALS, METHODS AND EXPERIMENTAL PROCEDURES

SELECTION OF LAGOON SITES AND LAGOON CONSTRUCTION

The sites selected for the two lagoons were at the Tulane University F. Edward Hebert Research Center in Belle Chasse, Louisiana and the Nueces County Sewage Treatment No. 5 in Port Aransas, Texas. These locations were chosen for the climatic and environmental conditions associated with each and their proximity to the research facilities at Tulane and the University of Texas Marine Science Institute at Port Aransas. The size and shape of the lagoons were determined after discussions with the EPA project officer, and it was decided that a lagoon with a capacity of about 7.5 cubic meters (approximately 2,000 gallons) would be large enough for the purpose of this study and small enough that obtaining the number of indicator organisms required for spiking each lagoon would be feasible. The lagoon construction was contracted to an experienced boatbuilder in Port Aransas who also had graduate training in marine science. The lagoon for each site was constructed of exterior grade 2 cm (3/4 in) thick plywood with the dimensions of 1.8 meters by 1.8 meters wide and 3.0 meters in depth (Figure 1). It was reinforced at the corners and around the width of the box with 5.0 by 10.0 centimeter boards. It was then sealed by covering it with fiberglass inside and out. The boxes were then allowed to cure for one month, then they were filled with water for two days to let any chemical by-products of the fiberglassing process leach out. They were then drained and prepared for transport to the final sites.

The boxes were then transported to the selected sites and installed under the supervision of members of the Tulane research team. The location of the lagoon at each of the sites is shown in Figure 2. Installation of the boxes was contracted out to local construction companies at each locality. A 3.0 meter deep hole with a 1.9 square meter area made with a backhoe and the box lowered into it with a crane. This was carried out under the supervision and assistance of Nueces County employees in Port Aransas and the Tulane Physical Plant employees at the Tulane University Hebert Research Center, Belle Chasse, Louisiana. In order to keep out humans and animals which could disrupt the project and to protect the public, each site was surrounded by a 2.4 meter high chain link fence topped with barbed wire. A 1.2 meter width of hail screen wire was attached to the bottom of the fence to provide protection from smaller animals. The hail fencing extended 0.46 meters below the ground to deter any burrowing animals. A screen cover was placed over each lagoon to prevent entry of any flying animals or insects.

Once the boxes were installed, they were filled with water to test for

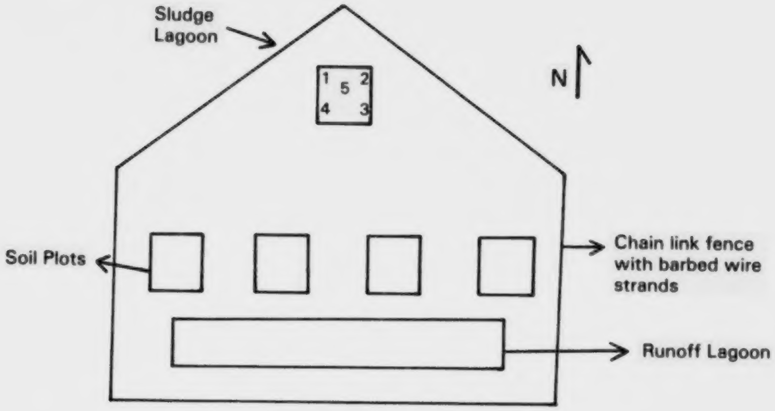

2"
seam
5/8" sheeting plywood
4"
10' - 0"
INSIDE VIEW
2"x4" nailed on flat
6' - 0"
2x4"
2"
seam
2x4" on flat
6' - 0"
2"
6' - 0"
TOP VIEW

NOTE: Box to be fiberglassed both inside and out.

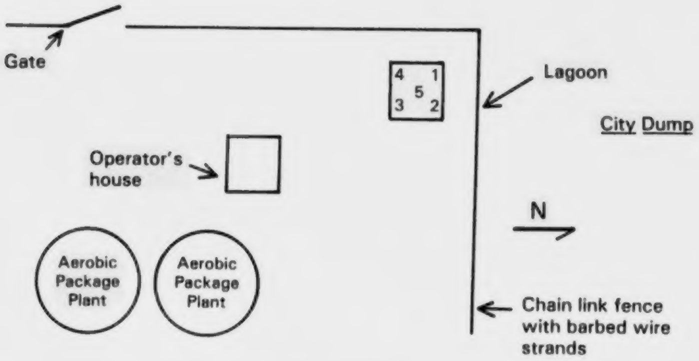
Two plain sides with seams attached to sides shown with 2"x4"s on seams.

Scale ½" = 1' - 0"

Figure 1. Open-top box design for EPA sludge lagoon.


Sludge Lagoon
1
5
2
4
3
N
Soil Plots
Chain link fence with barbed wire strands
Runoff Lagoon

New Orleans Lagoon


Gate
4
1
5
3
2
Lagoon
City Dump
Operator's house
N
Aerobic Package Plant
Aerobic Package Plant
Chain link fence with barbed wire strands

Port Aransas Lagoon

Figure 2. Site locations. (Numbers in lagoon indicate number and location of samples at each collection.)

leaks. Leaks were patched with fiberglass and checked again. They were then filled with anaerobic sludge to a depth of about 2.6 meters (eight feet). The New Orleans lagoon was filled on January 20, 1984 with approximately 7.56 cubic meters (2,000 gallons) of anaerobically digested sludge from the Camp Plaichet Treatment Plant located in Jefferson Parish, Louisiana, near New Orleans. The sludge was transported by a tank truck operated by Jefferson Parish. At the time the sludge was being placed in the lagoon, the spike mixture containing Salmonella livingstone, Ascaris suum eggs and Poliovirus Type I at concentrations listed in Table 9, was also added. The organisms were mixed into the sludge by adding the spike mixture as the sludge was pumped into the lagoon. The sludge was pumped into the lagoon with considerable force and this thoroughly mixed the indicator organisms with the sludge.

TABLE 9. APPROXIMATE DENSITY OF INDICATOR ORGANISMS INTRODUCED INTO NEW ORLEANS AND PORT ARANSAS LAGOONS

Volume	Organism	Density/Lagoon
4.5 liters	Salmonella	4.5×10^{11} organisms
20.0 liters	Ascaris	6×10^{7} eggs
0.5 liters	Poliovirus Type 1	5×10^{10} PFU

On February 23, 1984, approximately 7.56 cubic meters of anaerobically digested sludge from the Westside Treatment Facility at Corpus Christi, Texas was transported to the lagoon site at Port Aransas in a tank truck. At the same time that the sludge was pumped into the lagoon, the spike mixture with the same concentrations of organisms as used in the New Orleans lagoon was added using the same procedure. Initial samples were taken from each lagoon 24 hours after the indicator organisms had been added.

SAMPLING TECHNIQUES AND SCHEDULE

In order to characterize and quantify biotic and abiotic parameters in the lagoons, it was necessary to take representative samples of top and bottom components. The EPA project officer requested that five samples be taken at the top and five from the bottom levels. Sampling locations were at each corner of the box, one foot from each side and at a depth of 0.3 meters from the surface and 0.3 meters from the bottom, and two samples from the center of the box at the same depths (See Figure 2). Each sample was of a volume of one liter and was examined separately. Initial samples were taken 24 hours after spiking and then samples were taken at 1, 2, 3, 4, 6, 9, 15 and 24 months after spiking to monitor the survival of bacteria and viruses. For parasite analysis, samples were collected every three months for a period of two years. As each sample was collected, abiotic parameters dissolved oxygen, temperature, conductivity, oxidation, reduction potential (ORP) and pH were measured at the collection site and then again in the laboratory to detect any changes in the sludge characteristics between the collection and arrival at the laboratory. Additional abiotic parameters, as shown in Table 10, were also analyzed.

Prior to sample collection, water temperature and conductivity measurements were taken using a Salinity-Conductivity-Temperature meter (Yellow Springs Instruments, Model 33 S-C-T). Measurements were taken at the upper levels first to avoid disturbance of the sediment layer and then bottom measurements were taken.

At first, samples were collected using a Van Dorn sampler (Carolina Biological, No. 65125) for top and bottom samples taken at 24 hours and at all subsequent samplings of the top levels. Because of compaction, subsequent bottom samples were collected with a Van Dorn sampler as shown in Figure 3. As with on site abiotic measurements, top samples were taken first so as not to disturb the sediment layer. The samples were placed in sterile one liter Nalgene bottles (Nalge 2105).

Bottom samples were taken by removing a cork at the bottom of the Van Dorn sampler's PVC pipe and placing the pipe at a right angle to the sediment and then slowly lowering it into the lagoon so as to not disturb the settled sludge. After collecting the sediment, the corer was raised off the bottom and the rubber stopper was pulled home by means of a monofilament line. The pipe was then securely sealed by pushing the stoppered end against the lagoon bottom. The sampler was then removed and the sediment was transferred to a sterile one liter Nalgene bottle.

After all samples were obtained, Oxidation/Reduction Potential (ORP) and pH were determined using an Orion Model 399A Portable Specific Ion Meter. The samples were then taken to the laboratory for bacterial, viral and parasite analysis and additional abiotic characterization. Samples collected at the Port Aransas site were packed in ice and shipped to New Orleans via an overnight courier. Upon receipt in the laboratory, 100 mL aliquots of each sample were dispensed for bacterial and viral analyses and the remaining portion transported to the parasitology laboratory. The aliquot for virus analysis was placed in a small 100 mL plastic bottle and stored at -80°C. Samples for parasite analysis were stored at 4°C until examined, which was usually within a few days. Abiotic measurements and bacterial analyses were begun within 24 hours after collection.

SELECTION AND COLLECTION OF INDICATOR ORGANISMS

The microorganisms to be monitored were selected after consultation with the project officer and EPA researchers. It was decided that the enteric bacteria that normally occur in high levels in sludge, i.e.,

TABLE 10. ABIOTIC PARAMETERS MEASURED AND THEIR RESPECTIVE REFERENCES

Abiotic Parameter	Reference	Comments
Chemical Oxygen Demand (COD) mg/L	SM[a] Part 508C Closed Reflux Method (Hach)	Required some dilution
Solids measurement Total & Volatile %	SM Part 112	No problem
Ammonia mg/L as NH_3	SM Part 417A&D (Distillation) Part 417E (Selective Electrode)	Change to probe
Total Kjeldahl Nitrogen (TKN) mg/L	SM Part 420B (Semi-Micro)	Easy for waste sludges
Alkalinity mg/L as $CaCO_3$	SM Part 403	No problem
Hardness mg/L as $CaCO_3$	SM Part 424F (Hach)	Required an acid digestion
Nitrate mg/L as NO_3^-	SM Part 418C (Hach)	Must refrigerate
Nitrate mg/L as NO_3^-	SM Part 419 (Hach)	Must refrigerate
pH in standard units	ASTM[b] D 1293-78	No problem
ORP in ± millivolts	ASTM D 1498-76	Need to clean probe due to sulfide contamination
Conductivity (umho)	SM part 205	YSI probe used
Temperature (Deg.Celsius)	SM part 205	No problem
Dissolved Oxygen	SM part 431	Need to check membranes

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[a] Standard Method for the Examination of Water and Wastewater, 16th edition (39).

[b] 1980 Annual Book of American Society of Testing Materials Standards - Part 31 Water (40).

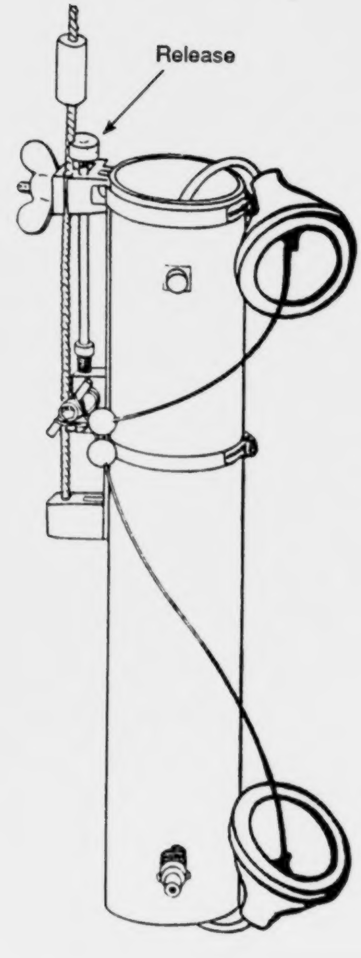

Figure 3. Sketch of Van Dorn Sampler

coliform bacteria and fecal streptococci, should be monitored as well as one pathogenic bacterium. The pathogenic bacterium selected was a strain of S. livingstone obtained from Dr. Albert D. Venosa, EPA-WERL, Cincinnati, Ohio. To prepare the S. livingstone for use as a spike for the lagoon sludge, cultures were grown on Xylose-Lysine-Deoxycholate Agar and then suspended in 4.5 L of Dulcitol Selenite Enrichment Broth at the concentration shown in Table 9 (4.5 x 10^{11} S. livingstone).

The virus chosen as an indicator of enterovirus survival in the stored sludge was the vaccine strain of Poliovirus Type 1. Polio 1 was selected because of its wide use for the monitoring of virus survival in sludges. The virus was obtained from the American Type Culture Collection, Rockville, MD., and replicated in African Green Monkey kidney cell cultures. The virus produced from these cultures was titered and then stored at -80°C until used (Appendix A).

The parasite chosen to be monitored in the stored sludge was Ascaris suum. The eggs of Ascaris spp. have been widely used in inactivation studies because they are among the most resistant forms of pathogens known, are easily recovered and identified, and can be obtained in relatively large numbers. In addition, the parasite can cause disease in man and animals. The eggs of Ascaris suum, the pig parasite, were chosen rather than those of Ascaris lumbricoides, the human parasite, because the former are more readily available in the United States. However, the logistics of obtaining the large numbers of eggs required for the spiking of the two lagoons, each containing 2,000 gallons, presented problems. We decided to obtain the A. suum eggs from naturally infected pigs on pig farms. Attempts were made to find localities where the feces of infected pigs could be obtained. Dr. Blair Leftwich, Lubbock, Texas, was contracted to supply the A. suum eggs. The fecal droppings of pigs on a farm near Lubbock were collected by washing them from the floor of concrete holding pens and then placing the collected material in large containers to allow settling. After one day, the supernatant was discarded and the sediment transported to the laboratory where it was passed through sieves to remove the larger particles. The remaining material was centrifuged at 800g for 15 minutes and the supernatant discarded. The packed sediment was stored at 4°C. After several weeks of collecting to obtain the desired number of eggs, the pooled sediment was packed in ice and sent by overnight courier to the laboratory at Tulane. The sediment of the washed pig feces was then re-suspended in water and passed through finer sieves. The suspension was centrifuged and the resulting sediment mixed with a $MgSO_4$ solution (specific gravity of 1.20) to float and separate the eggs from the other particles. After centrifugation at 800g for 5 minutes, the floating material was collected, diluted in water and re-sedimented by the same centrifugation procedure. The resulting sediment, which contained the concentrated Ascaris eggs, was stored at 4°C until it was processed through bench-scale anaerobic digesters.

The A. suum eggs were added to fresh municipal sludge and treated with an anaerobic digestion process so they would be exposed to similar conditions to which eggs occurring naturally in sludge would have been exposed. The bench-scale digester process is described in a following section. After a period of 14 days, the eggs were removed and used for spiking the lagoon-stored sludge. The number of eggs spiked into each lagoon is shown in Table 9.

BENCH-SCALE ANAEROBIC DIGESTERS

Two bench-scale digesters used to run continuous anaerobic digestion experiments were constructed of a 45.7 cm length of Plexiglas tubing that had an inside diameter of 35.4 cm and a wall thickness of 9.5 mm. Each was totally enclosed and had a capacity of 15 liters. Complete and constant mixing of the sludge in the digester was accomplished with a fan-cooled, heavy duty stirrer with two propellers on the shaft. Temperature in the digester was controlled to about 35°C by using a 1.82 m by 25.4 mm heating tape connected in series to a rheostat and a controller which was, in turn, connected to a probe in the digester chamber. Anaerobic conditions were maintained by sparging nitrogen from a compressed gas cylinder through a 15.2 cm airstone in the digester. A 75 mL Kjeldahl bulb was used to condense water vapor to reduce the loss of water due to evaporation. A lead acetate bubbler followed the Kjeldahl bulb to trap H_2S in the gases produced during the digestion process (39). This apparatus is shown in Figure 4.

Sludge from an operating anaerobic digester at a wastewater treatment plant in Jefferson Parish, Louisiana, utilized as a feed, was placed into each digester at the beginning and then 1.5 liters of a mixture of primary and returned activated sludge (equal volumes) from another plant in Jefferson Parish was added to each digester every other day. An equal volume was withdrawn from the digester prior to the addition of the feed sludge. A 15-day hydraulic detention time was attained.

Sludge was monitored for temperature, gas production, suspended solids, volatile suspended solids, pH and total organic acids using Standard Methods procedure (39). The digesters were run until they reached equilibrium, and they were spiked with only the parasites since the viral and bacterial pathogens are susceptible to inactivation by anaerobic digestion.

Washed pig feces containing concentrated *A. suum* eggs were added with the feed sludge after the digestion process had stabilized over a period of several weeks. Subsequently, all sludge removed from the digester and all of the sludge remaining in the digester at the end of the digestion period was stored at 4°C until used as the *Ascaris* egg spike for the sludge placed in the lagoons. The initial number and viability of the eggs in the spike was determined at time of spiking.

ANALYTICAL PROCEDURES

Bacteria

Upon arrival of the lagoon samples at the laboratory, each was homogenized using a Waring blender to break up large particulate matter. An amount of 30 g to 50 g of sludge was homogenized in 200 mL of water at

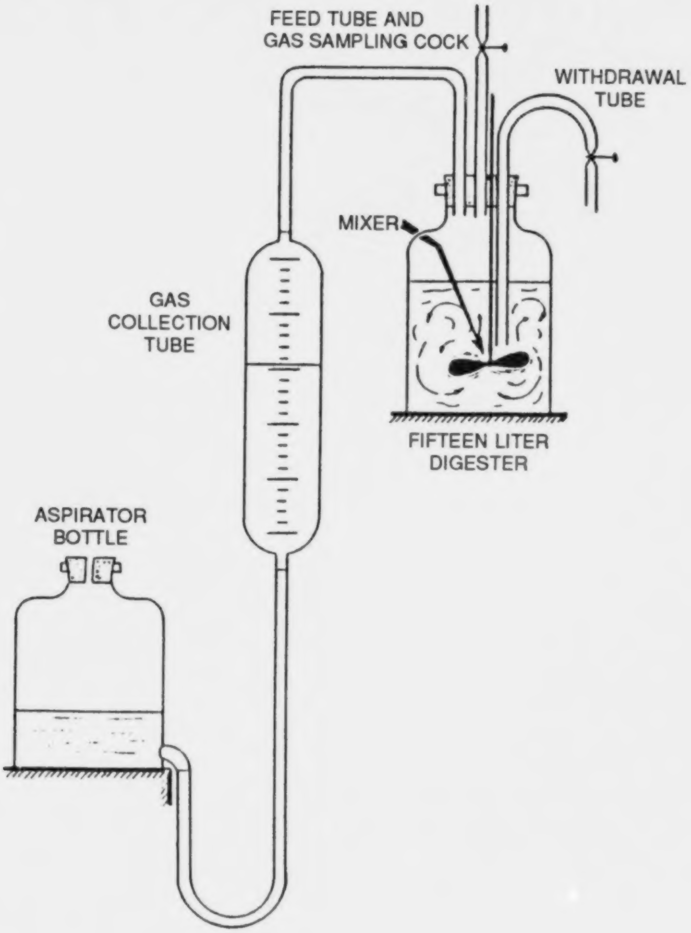
FEED TUBE AND
GAS SAMPLING COCK
WITHDRAWAL
TUBE
MIXER
GAS
COLLECTION
TUBE
FIFTEEN LITER
DIGESTER
ASPIRATOR
BOTTLE

Figure 4. Schematic layout of fifteen liter batch fed digester

high speed for one minute with that blender (Blender 700 Model 33 BL 79). Quantification of total coliforms, fecal coliforms, and fecal streptococci was done using the multiple tube fermentation procedure included in Standard Methods, 16th Edition (39), for coliforms and streptococci enumeration (Table 11). Salmonella livingstone as well as Citrobacter freundii-like organisms enumeration was done by the modified Kenner and Clark method (41).

Viruses

The methodology for the determination of enteroviruses in the sludges followed the method described by Berman et al. (42). Virus inactivation was determined by the presence or absence of quantifiable plaques formed on BGM cell monolayers following the inoculation of material eluted and concentrated from the sludge samples. BGM cell cultures were prepared in accordance with prescribed EPA Laboratory Methods. The complete procedure for viral analysis is described in Appendix A.

Parasites

The initial samples taken from the New Orleans and the Port Aransas lagoons were examined by Procedure A, Appendix B. In this procedure, the floating material recovered after the sludge had been mixed with the $MgSO_4$ flotation solution was subjected to an additional concentration procedure to separate as much of the particulate matter from the Ascaris eggs as possible. The purpose of this was to reduce the amount of sediment that had to be examined microscopically after culturing. This additional procedure utilized an acid-ether centrifugal sedimentation step, and following the examination of the initial lagoon samples, it was discovered that the procedure affected the ability of the recovered Ascaris eggs to develop to the infective larval stage in cultures.

Procedure B (Appendix B) was then developed which eliminated the acid-ether step. In Procedure B, the upper portion of the supernatant in the tubes following the $MgSO_4$ flotation step was poured through a 400 mesh (38 cm) sieve which caught the Ascaris eggs. The material caught on the sieve was then concentrated by washing it off the sieve and then centrifuging it. This material was then placed in culture to determine viability of the recovered eggs. This procedure was shown to have no effect on the ability of Ascaris eggs to subsequently develop to the infective stage in culture.

The determination of the number of Ascaris eggs present in the sludge processed through the bench-scale anaerobic digesters was made by using a dilution-aliquot procedure. A measured portion of the sludge was diluted to a known volume with water and then three or more aliquots of 0.075 mL each were examined microscopically and the number of eggs counted. The number of eggs per milliliter was then calculated using the dilution factors. Eggs in the samples were then placed in culture to determine the viability of the eggs following the method given in Procedure B.

TABLE 11. LIST OF MEDIA AND METHODS USED FOR BACTERIA ANALYSIS

Organism	Incubation Media [Temperature C]	Reference
Total Coliform[a]	Lauryl Tryptose [37°C]	SM[b] Parts 908A, 909A
Fecal Coliform[a]	Lauryl Tryptose/EC Media [37°C/44.5 ± 0.2°C]	SM Parts 908C, 909C
Fecal Streptococci[a]	Azide Dextrose Broth / Bile Esculin Azide Agar [35°C/35°C]	SM Parts 910 A & B
Salmonella livingstone	Dulcitol Selenite Enrichment Broth/Xylose Lysine Deoxycholate Agar/Triple Sugar Iron Agar [40°C/37°C/37°C]	Kenner and Clark Method for *Salmonella* Enumeration (41)

[a] The initial membrane filtration technique (which was determined for project) had to be changed to MFT technique (reference above) due to high solids and particulate matter in samples.

[b] *Standard Methods for the Examination of Water and Wastewater*, 16th edition (39).

Abiotics

Certain abiotic parameters were measured to determine their influence on the survival of the pathogenic microorganisms. The parameters measured and the reference to the procedures used are listed in Table 9.

Controls for Parasites and Viruses

In order to have temperature controls for *Ascaris* eggs and Poliovirus Type 1 that would exclude the affects of the sludge environment, suspensions of these microbes were placed in vials containing distilled water, or in the original growth medium in the case of the virus. The vials were then submerged in the lagoon-stored sludge. *Ascaris* eggs were from the same stock of eggs used as the spikes. Distilled water was deoxygenated by heating it to the boiling point, then after it had cooled, 1 ppm of sodium sulfite was added to scavenge any remaining oxygen. The eggs were washed once in the deoxygenated water, then approximately 2,000 eggs were distributed to each of twenty 20 mL screw-cap glass tubes, deoxygenated water was added to the top and the tubes sealed. For the virus controls, Poliovirus was diluted to 10^4PFU/mL and 0.5 mL placed in each of twenty 1 mL Nalgene vials.

Ten vials of each microbe were placed in each of two one-quart Thermos bottles, gravel was placed in the bottom of each, and a cord was attached. A bottle was lowered to the bottom of each lagoon.

When two large Plexiglas columns were filled with spiked sludge on July 16, 1985, distilled water controls were also initiated. Thirteen tubes with Ascaris eggs were placed in each column. Distilled water was deoxygenated by boiling, but in this case no sodium sulfite was added. Eggs from the same stock as used to spike the sludge in the columns were washed in the deoxygenated water and then, about 1,500 Ascaris eggs were transferred to each of twenty-six 5 mL screw-cap tubes. Each tube was filled to the lip with the deoxygenated water, tightly sealed with paraffin and then capped. The tubes were then placed in a one liter Nalgene® bottle, the bottle filled with distilled water, and the bottle submerged in the sludge in the same manner as the controls were placed in the lagoon. Virus controls were not placed in these columns.

In discussions with the project officer, it was decided to place in each lagoon a plastic tube that would be filled with sludge that was spiked with a much higher concentration of Ascaris eggs than that spiked into the lagooned sludge. The purpose of having a small volume of sludge with a high concentration of eggs was to be able to detect a greater magnitude of die-off of eggs than would be possible in the spiked sludge in the lagoon. Each column was constructed of a 2.4 meter (8 ft) length of 10.2 cm (4 in) diameter PVC tube that was fitted with a cap at the bottom end. The tube was lowered into the lagoon and anchored to the sides of the box at one corner. For the New Orleans lagoon, 0.5 L of the stock spike mixture from the digester was mixed with 19.5 L of same sludge that was placed in the lagoon. This spiked sludge was then used to fill the PVC tube. The same procedure was to be used for the tube in the Port Aransas lagoon; however, the initial samples from this tube indicated that the egg densities were no higher than those of the sludge from the lagoon. This indicated that the Ascaris egg spike may inadvertently not have been mixed into the sludge added to this tube.

Quality Assurance

Strict quality assurance methods were carried out in this project so that the laboratory analyses could be shown to be both accurate and precise. In the field of wastewater analysis, this is especially important due to physical and chemical properties of sludges that may affect the analyses. For example, it was necessary to initially measure ammonia concentration in the lagoon samples using the ammonia distillation method due to the presence of surfactants in the sludge that interfered with the ammonia electrode membrane. This was apparent when the quality assurance samples were analyzed because the recovery values were lower than expected. After sufficient destruction of the surfactants had occurred over time in the lagooned sludge, the ion selective electrode method was then suitable for ammonia determination.

The quality of abiotic (except COD and nitrogen) and bacterial (except Salmonella) enumeration techniques was determined by analyzing a duplicate and a spiked subsample on every fifth sample received. In the Salmonella, parasite and virus analyses, duplicate and spiked subsamples were analyzed on every tenth sample received. Duplicate sample analysis was used to determine the precision of the methods, while the addition of a known concentration of the substance being measured, be it Salmonella organism, chemical oxygen demand, or Ascaris eggs, determined the accuracy of the methods. Every sixth month, the abiotic analytic procedures were checked by the examination of a set of known test samples to determine the concentration of abiotic constituents; this was part of EPA's Laboratory Performance Evaluation.

The calculation of standard deviation and percent recovery was performed on the abiotic measurements, i.e., COD, NH_3, and TKN. For COD measurements, the spike was 50 mg of dextrose added to 100 mL of the sample. With ammonia and TKN measurements, the spike was 10 mL of a 1000 ppm ammonium chloride standard solution. Replicates were considered to be within QA guidelines if they differed by less than one unit of standard deviation. The results of spiked samples were not considered acceptable if their recovery was less than 70% or greater than 120%. All analyses performed were within EPA guidelines or were repeated to determine any source of error. Quality assurance data are listed in Appendix C.

Because it was evident that the sludge received from the Corpus Christi plant contained high levels of refinery wastes, these sludges were analyzed for petroleum constituents. The initial analyses were conducted by standard gas chromatographic procedures for crude oil in soils and sludges. Subsequently, sludge from this plant was again analyzed by GC/MS and the supernatant was analyzed for the 95 priority hazardous waste constituents as well as for an additional 500 listed hazardous compounds. (The initial samples were analyzed by Coast Science, Inc., Austin, Texas, and the second samples were analyzed by Environmental Industrial Research Associates, Inc., St. Rose, La.) The procedures used were as follows: water - EPA Methods 624 and 635 (Volatile and Semivolatile Organics by GC/MS); sludge - SW Method 8240 (Solid Waste by GC/MS) found in Standard Methods (39).

LARGE COLUMNS FOR SLUDGE STORAGE

After it was observed that the Ascaris eggs in the sludge in the Port Aransas lagoon had died off earlier than those in the New Orleans lagoon, it was decided to initiate another study in which sludge from the same sources would be spiked and placed in large columns rather than in lagoons because of the cost of constructing other lagoons. Two columns were constructed; each was a 3.66 m (12 ft) length of a Plexiglas cylinder with an inside diameter of 0.3 meters (1 ft.). A 0.91 x 0.91 m (3 x 3 ft) piece of flat Plexiglas was attached to one end with epoxy glue. The two columns were placed side by side in a shady area outside the laboratory at Tulane's Hebert Research Center near the New Orleans lagoon. The columns were chained to the wall of the laboratory to prevent them from being tipped over. In each cylinder, sampling ports were constructed by drilling a hole through the cylinder at each port site and inserting a piece of plastic tubing through the hole until it extended 7.5 cm (5 in) into the cylinder. The tubing was sealed to the wall of the cylinder with epoxy cement. A screw clamp was used to close the outer end of the tubing. Sampling ports were located 0.3 m (1 ft) and 2.13 m (7 ft) from the bottom of each cylinder. Each cylinder was wrapped with a 7.6 cm (3 in) thick fiberglass blanket to serve as insulation. Each cylinder was fitted with a cap.

To prepare the egg spike for these columns, approximately 9 x 10^6 Ascaris suum eggs collected in April, 1985 from naturally infected pigs in Lubbock, Texas, by the procedures described elsewhere, were added to sludge in a bench-scale anaerobic digester. The anaerobic digestion was carried out over a two week period following the procedure described previously. The spiked sludge was then divided into two equal volumes and each was stored at 4°C until added to the sludge placed in the columns.

In July, 1985, about 379 L (100 gal) of domestic sludge was obtained from each of the plants that were the source of the sludge used to fill the New Orleans and the Port Aransas lagoon, respectively. On July 16, 1985, sludge was added to each column to a depth of 2.4 m (8 ft) or a total of 178 L (47 gal). While the sludge was pumped into the column, the egg spike was added to obtain proper mixing of the Ascaris eggs in the sludge.

Samples were taken immediately after the spiked sludge was added and then 1 month, 3 months, and subsequently every third month until viable Ascaris eggs were not recovered for at least two consecutive sampling periods. A sample of 500 mL was collected from each sampling port at each collection time and a distilled water control vial was removed at the same time. The sludge samples were examined for parasite eggs following Procedure B (Appendix B). The suspended solids were also measured.

SLUDGE SETTLING EXPERIMENT

In order to determine the rate of settling of Ascaris eggs in anaerobically digested sludge, an anaerobic sludge of about 4% suspended solids and containing A. suum eggs was placed in a Plexiglas tube, 1.8 m (6 ft) high and 18 cm (7 in) in diameter. The tube had sampling ports at 0.3 m (1 ft) intervals from one located at the bottom. A. suum eggs recovered from the feces of infected pigs were added to the sludge, the sludge mixed thoroughly, and then the sludge was added to the tube to a depth of 1.83 m (6 ft). Immediately, a sample of approximately 50 mL was drawn from each port; similar samples were subsequently taken at 3, 6, 12 and 24 hours, 1 week, and 1, 2, and 3 months after the start of the experiment. The samples were analyzed for suspended solids and the density of the Ascaris eggs. For samples that had suspended solids of much below 4%, the samples were mixed thoroughly and three 1 mL subsamples withdrawn. Each of these was centrifuged and then the entire sediment of each was examined under the microscope. For the denser samples, after three 1 mL subsamples were withdrawn, each subsample was diluted to 5 mL in water and then, after mixing, three 1 mL aliquot samples were withdrawn. Each of these was centrifuged and the sediment was then examined under the microscope and the Ascaris eggs counted.

DATA STORAGE AND ANALYSIS

The Tulane University computer system (DEC 2060) was used for data storage and analysis. The BMDP.2R (Biomedical Data Processing) computer program and Statistix (SX) were used for the analysis of the data (43, 44). Descriptive statistics as well as regression analysis were performed in order to determine what effects, if any, abiotic parameters could have on the viability of the microorganisms being monitored. Two types of regression analyses were done on the aqueous and sediment data from two lagoons. Each data set consisted of nine abiotic and six biological parameters taken on ten to twelve separate occasions. The raw abiotic data were derived from this study, and arithmetic means were calculated for daily sets of readings. The other five biological parameters (total coliform, fecal coliform, fecal streptococci, *Salmonella*, Poliovirus) were expressed as geometric means. The rates of inactivation were calculated using the first order kinetics model (45), while for *Ascaris* eggs, only the percent inactivation over time was calculated. Also, the regression analysis was made for the log of each biological parameter (except for *Ascaris* where actual values were used) versus each abiotic parameter for all lagoons and columns. The computer F values were converted to the smallest significant incremental P value to which it could correspond.

The viability or non-viability of *Ascaris* eggs was determined on the basis of egg culture results (larval development).

The overall mean percent of inactivation of *Ascaris* eggs was calculated according to the formula:

initial % viability - final % viability = % Inactivation

SECTION 6

RESULTS

Results are presented in the order of abiotic and climate data, anaerobic digester data, organics, bacterial survival, virus survival, parasite survival and statistical analysis. The first three sections deal with pretreatment of the Ascaris eggs and accumulation of the necessary data to properly evaluate the lagoon storage system. The next three involve the bioassay for parasites, bacteria and viruses. The final section includes a statistical analysis of the survival data.

ABIOTIC AND CLIMATIC DATA

The abiotic data are shown in Appendix C, and the climatic data for New Orleans are compiled in Appendix D. The Port Aransas climatic data can be found in local climatical monthly summary data for NOAA (National Oceanic and Atmospheric Administration), Corpus Christi, Texas, International Airport from January, 1983 to September, 1986. In addition, the required quality assurances are also included in Tables C-21 and C-22, Appendix C. The New Orleans lagoon exhibited the typical seasonal fluctuations, as noted by a drop in COD, increase of nitrates and nitrites and rise of the conductivity during the winter-spring period, which indicates the feedback of oxygen demand from sludge during warm periods in the New Orleans area (See Table 12).

The temperature of sludge in the New Orleans and Port Aransas lagoons at time of each sampling is shown in Figure 5. In the New Orleans lagoon, the temperatures ranged from a low of about 11°C in February 1985 to a high of 27°C in August 1985. In the Port Aransas lagoon temperatures ranged from a high of 29°C in July 1984 to a low of about 9°C in January 1985.

The temperature of sludge stored in the two large columns at time of each sampling is shown in Table 13. The temperatures ranged from a high of 26-27°C in the summer months to a low of 17°C in January 1986.

ANAEROBIC DIGESTION

The digesters were started up 3 months before spiking. The first digesters were spiked with Ascaris eggs on October 17, 1983. After ten days, the two digesters were stopped and the spiked sludge stored in the refrigerator for later spiking of the lagoons. The operational data is shown in Appendix E. The data on the numbers of Ascaris eggs and their viability are presented in Tables 14 and 15. In order to provide an Ascaris egg spike for the sludge stored in large plastic columns, the digesters were again started up in early 1985. The Ascaris egg data on this digester are shown in Table 16.

TABLE 12. ABIOTIC DATA FOR AQUEOUS PORTION OF LAGOON SLUDGE

	New Orleans 7/15/85	New Orleans 1/29/86
Temperature (^{o}C)	26	20
Dissolved Oxygen (mg/L)	5.6	7.4
pH (std. units)	7.6	6.9
Akalinity (mg/L as $CaCO_3$)	334	280
Hardness (mg/L as $CaCO_3$)	136	168
Ammonia (mg/L as N)	21	18
Nitrate Nitrogen (mg/L NO_3)	4.0	8.7
Nitrite Nitrogen (mg/L NO_2)	0.13	0.9
Total Phosphorus (mg/L PO_4)	20	19.5
Chemical Oxygen Demand (mg/L)	250	75
Conductivity (umhos)	95	382

TABLE 13. TEMPERATURE OF SLUDGE STORED IN LARGE COLUMNS

Type of Sludge	Storage time in months after project initiation (Month of year)							
	0 (JUL)	1 (AUG) 1985	3.3 (OCT)	6.4 (JAN)	9.0 (APR)	12.0 (JUL) 1986	15.2 (OCT)	18.5 (JAN)
New Orleans	26°C	26°C	25°C	20°C	26°C	27°C	19°C	17°C
Port Aransas	26°C	26°C	25°C	20°C	26°C	26°C	19°C	17°C

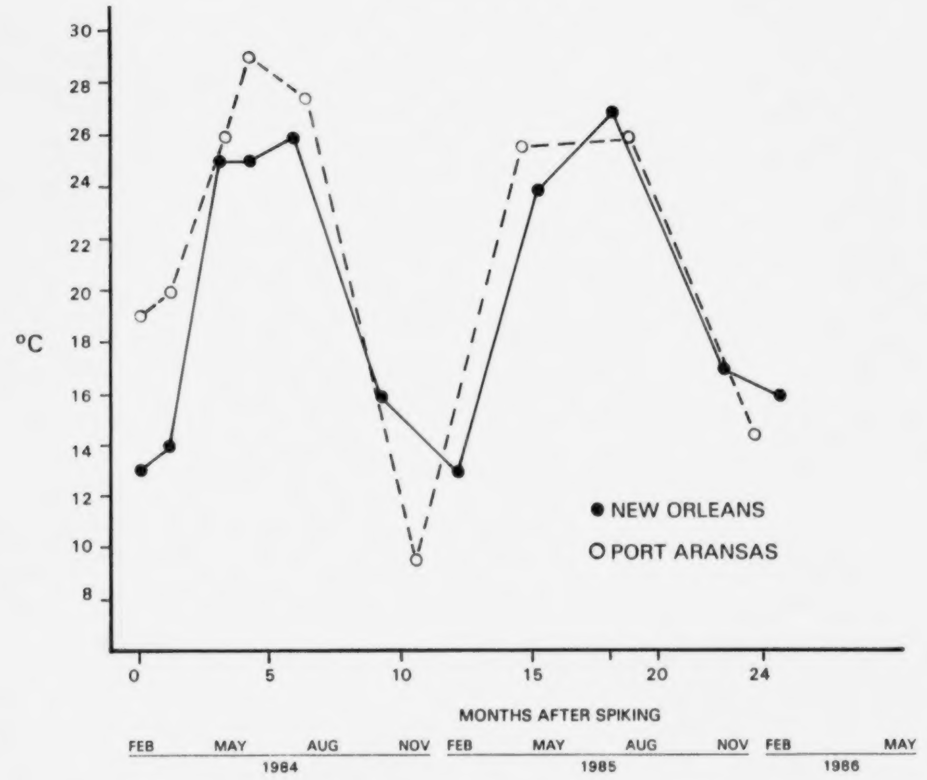

Figure 5. Temperature of sludge in New Orleans and Port Aransas lagoons at times of sampling.

TABLE 14. RESULTS OF PARASITOLOGIC ANALYSIS OF SLUDGE SAMPLES FROM ANAEROBIC DIGESTER NUMBER 1

DATE	Total number of *Ascaris* Eggs	% viable in culture	Total number of viable eggs/mL
18 Oct 1983	10,880	29.5	3,210
21 Oct	15,840	37.5	5,940
24 Oct	11,840	23.8	2,818
27 Oct	9,520	25.6	2,437
28 Oct[a]	10,100	22.7	2,293
28 Oct[b]	8,350	24.3	2,029

[a] Combined with sample from Digester Number 2; removed at time of feeding.

[b] Combined with sample from Digester Number 2; removed after feeding when digesters were stopped.

TABLE 15. RESULTS OF PARASITOLOGIC ANALYSIS OF SLUDGE SAMPLES FROM ANAEROBIC DIGESTER NUMBER 2

DATE	Total number of *Ascaris* Eggs	% viable in culture	Total number of viable eggs/mL
18 Oct	9,700	24.8	2,406
21 Oct	14,800	23.3	3,448
24 Oct	10,560	22.7	2,397
27 Oct[a]	10,800	22.5	2,430

[a] Combined with samples from Digester Number 1; see Table 13

TABLE 16. RESULTS OF PARASITOLOGIC ANALYSIS OF ANAEROBICALLY DIGESTED SLUDGE SPIKE FOR LARGE PLASTIC COLUMNS [a]

Incubation Period in Digester	Total number of *Ascaris* eggs/mL	% viable after culture	Total no. viable eggs/mL
14 days	450	90.6%	408

[a] *Ascaris* eggs spike added to anaerobic digester, incubated 14 days, then stored at 4°C until spiking of sludge in column

ORGANICS

Because of the unusual nature of the sludge in the Port Aransas lagoon, it was analyzed for petroleum organics. Table 17 shows the results of hexane/silica gel extraction, which quantified the extractable organics and separated them into three groups, saturated organics, aromatics and polar aromatics. These data indicate that the Corpus Christi sludge was composed of about 20 percent petroleum organics (by weight). Gas chromatographic analyses were run on typical crude oil, the Port Aransas lagoon supernatant water and the Port Aransas sludge. Figures 6 to 10 show these data for both saturated aliphatic and aromatic petroleum compounds found in crude oil. Upon the initiation of the column studies, GC/MS analyses for hazardous petroleum organics constituents were conducted on sludge from the Port Aransas and New Orleans lagoons. The Port Aransas' sludge had concentrations at least 3 orders of magnitude greater than the New Orleans' sludges, as noted in Table 18.

TABLE 17. GROSS ANALYSIS OF PETROLEUM ORGANICS IN THE PORT ARANSAS SUPERNATANT WATERS AND SLUDGES IN THE TEXAS FIELD LAGOON

Constituent	Sludge	Supernatant Water
Nature of Material	Heavy Black Sludge	Contaminated Water
Extractable Organics (gram/kilogram)	195.0	0.03
Saturated Aliphatics[a]	60.5%	52.4%
Aromatics[a]	19.5%	14.3%
Polar Aliphatics[a]	20.0%	33.3%

[a] Extracted with hexane through silica gel. This extraction assumes the percent fraction of the extractable organics to be 100 percent.

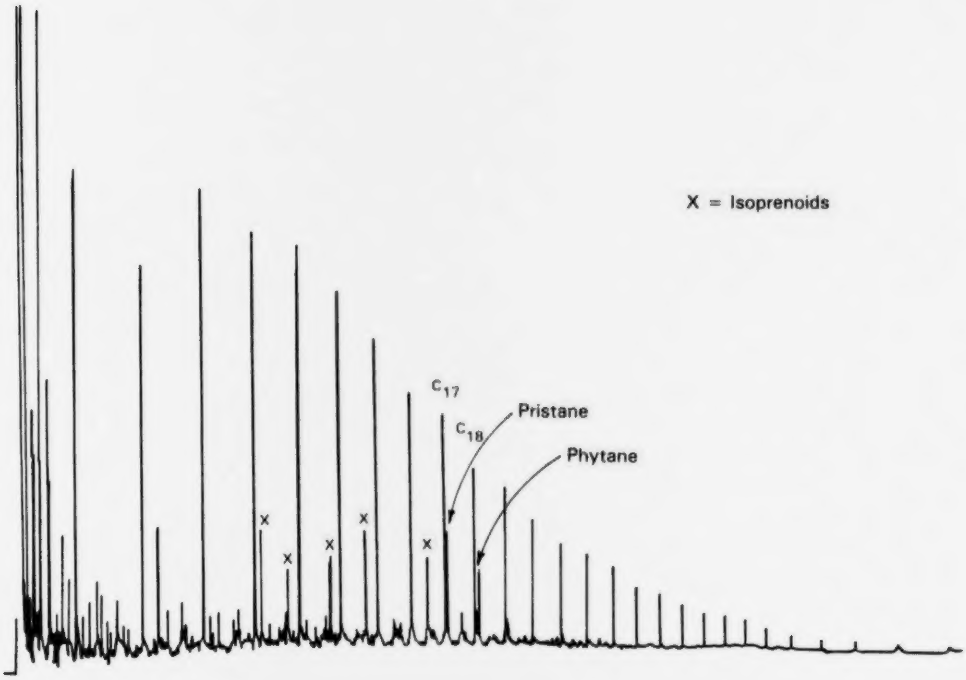

Figure 6. Chromatograph of typical crude oil for saturated organic compounds.

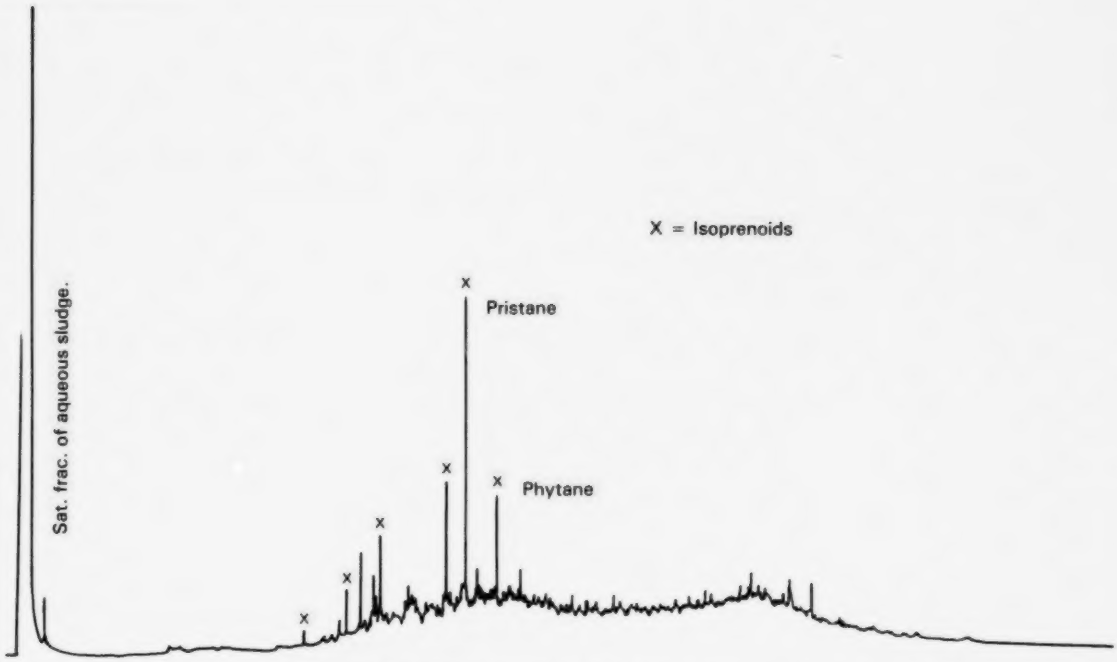

Figure 7. Chromatograph of the aqueous phase of the Port Aransas sludge for saturated aliphatic organics.

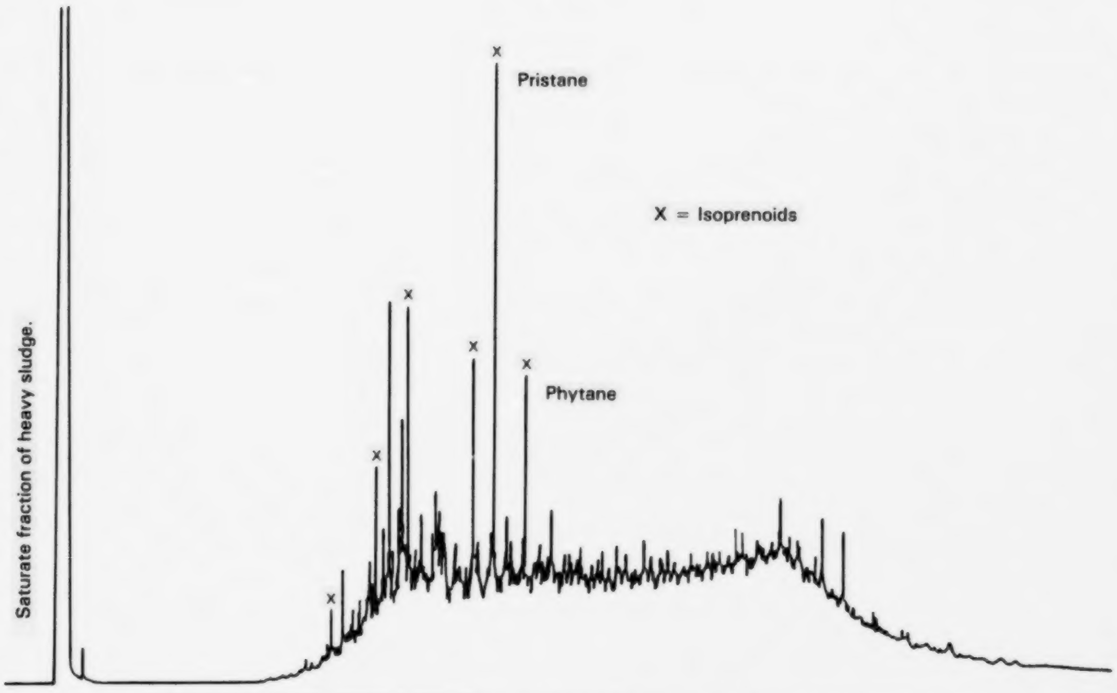

Figure 8. Chromatograph of Port Aransas sludge for saturated organics.

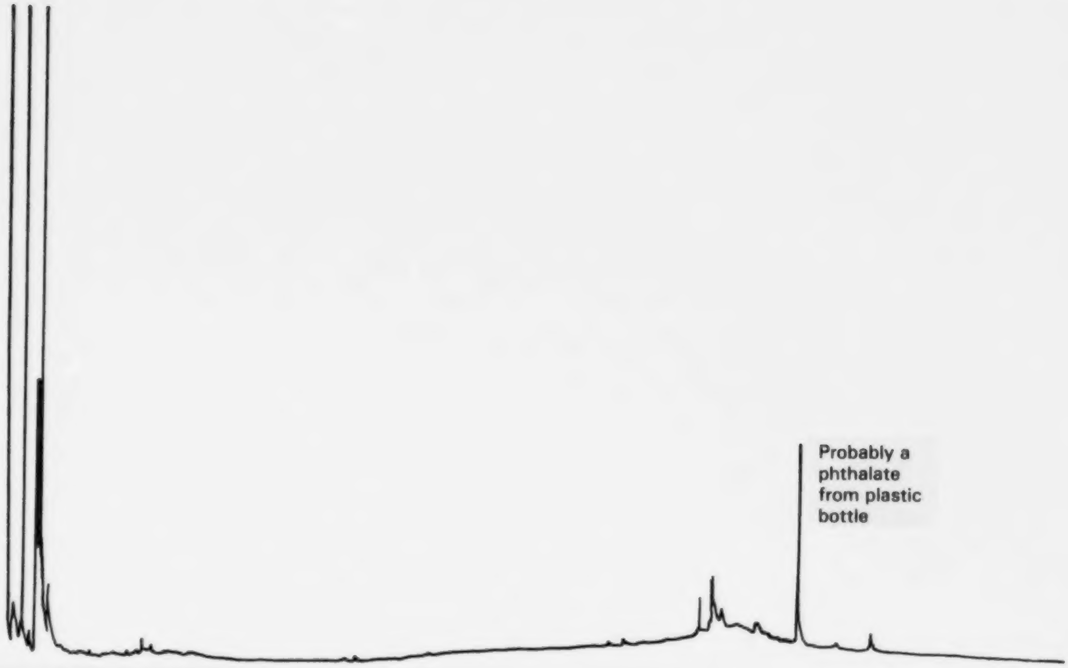

Figure 9. Chromatograph of the aqueous phase of the Port Aransas sludge for aromatic organics.

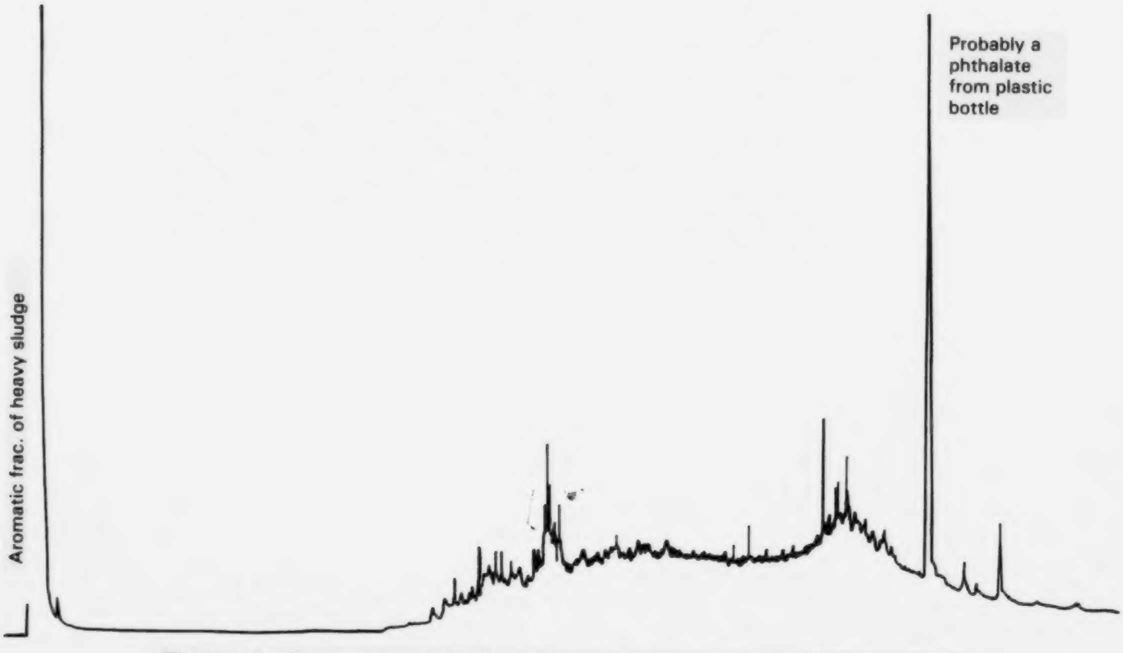

Figure 10. Chromatograph of Port Aransas sludge for aromatic organics.

TABLE 18. SUMMARY OF THE RESULTS OF THE GC/MS ANALYSES FOR ORGANICS IN SLUDGES STORED IN LARGE COLUMNS

Constituent	Port Aransas	New Orleans
Sludges (ppm)		
Naphthalene	5.0[a], 5.0[a]	<0.005
Phenanthrene	8.0[a], 9.0[a]	0.009[a]
Bis (2-ethylhexyl) phthlate	46.0, 47.0	<0.005
2-Methylnaphthalene	25.0, 28.0	0.0042[a]
1-Methylnaphthalene	17.0, 17.0	0.0025[a]
2,6,10,14-Tetramethypentadecane	190.0, 152.0	0.0231
Toluene	<10.0	0.0085[a]
1,4 Dichlorobenzene	<10.0	0.0025[a]
Pyrene	<10.0	0.0049[a]
Flueranthrene	<10.0	0.0058[a]
4-Chloroquiline	<10.0	0.0088[a]
Supernatant Waters (ppb)		
Ethylbenzene	5.3[a]	<10.0
Xylene	41.3	<10.0
1,4 Dichlorobenzene	2.8[a]	<5.0
1,2 Dichlorobenzene	4.3[a]	<5.0
Naphthalene	8.0	<5.0
Phenanthrene	5.2	<5.0
4-Chloroaniline	32.6	12.1
2-Methylnaphthalene	23.4	<5.0
2,6,10,14-Tetramethylpentadecane	44.6	<5.0
Phenol	13.8	<5.0
2,4-Dimethylphenol	22.6	<5.0
4-Methylphenol	3.1[a]	<5.0
1-Methylnaphthalene	17.2	<5.0

[a] Below quantifiable detection limits but detected at these concentrations

BACTERIAL SURVIVAL

The results of the bacterial analyses are shown in Figures 11 to 18. The rate of die-off of the indicator bacteria was estimated using the first order kinetic model (43). Rates of 0.18 log of MPN'S per 100 mL/month was observed for total coliforms in the Port Aransas sludges. The total coliform die off leveled off within ten months and showed a slight increase after 23 months of storage, suggesting a contamination of the site by other organisms, e.g., the insects which invaded the lagoon. Nonetheless, a regrowth pattern may be suggested as regards to fecal streptococci in both

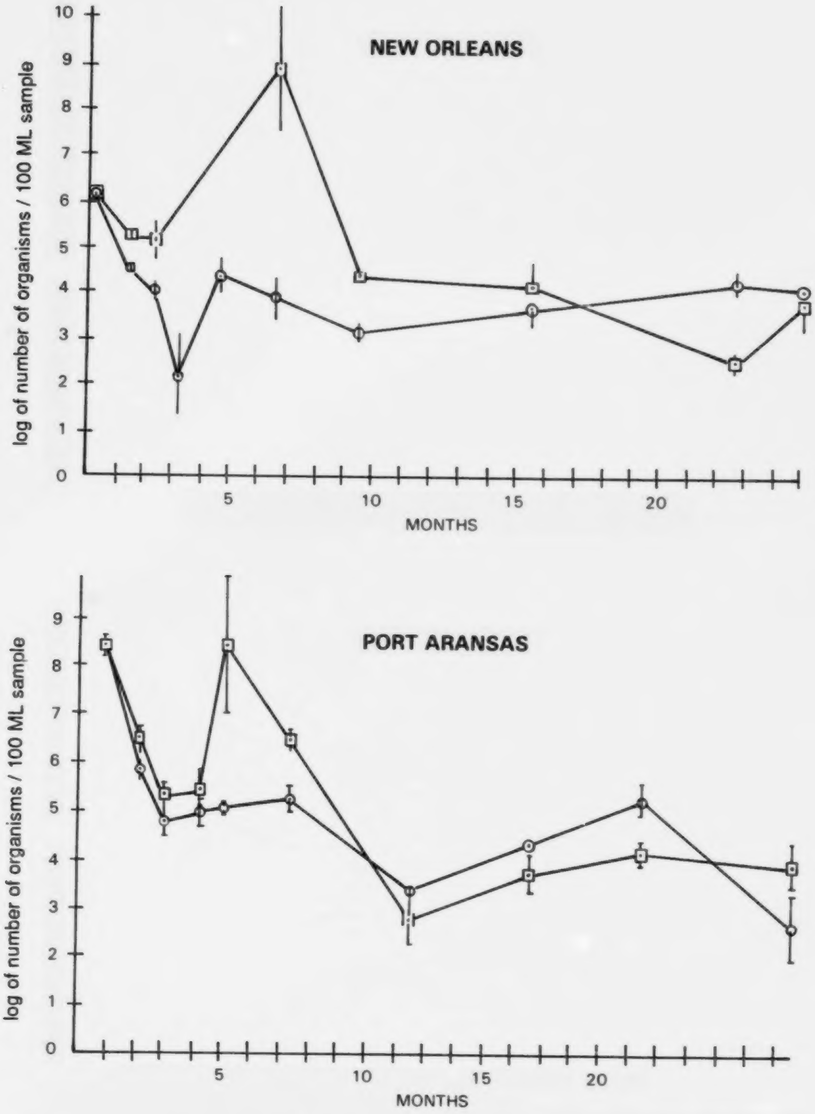

Figure 11. Graph of total coliform organisms per 100 milliliter versus time in months for New Orleans and Port Aransas sludge lagoons.

○ aqueous fraction
□ sediment fraction

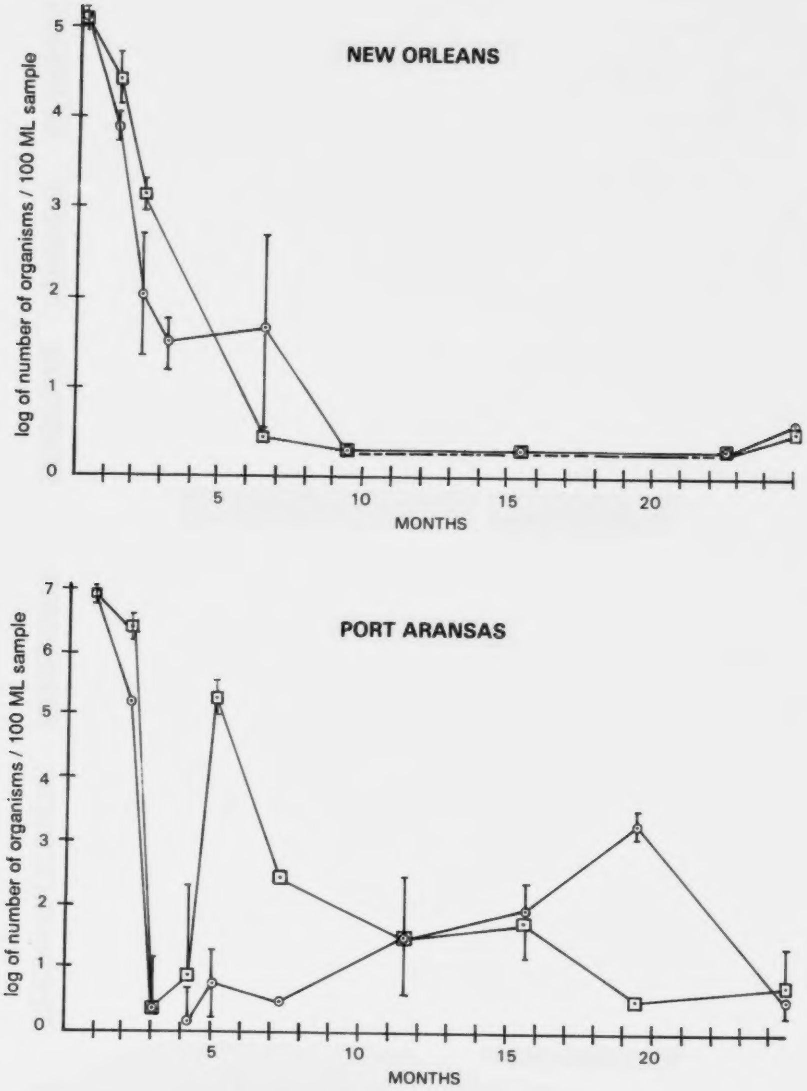

Figure 12. Graph of fecal coliform organisms per 100 milliliter versus time in months for New Orleans and Port Aransas sludge lagoons.

○ aqueous fraction
☐ sediment fraction

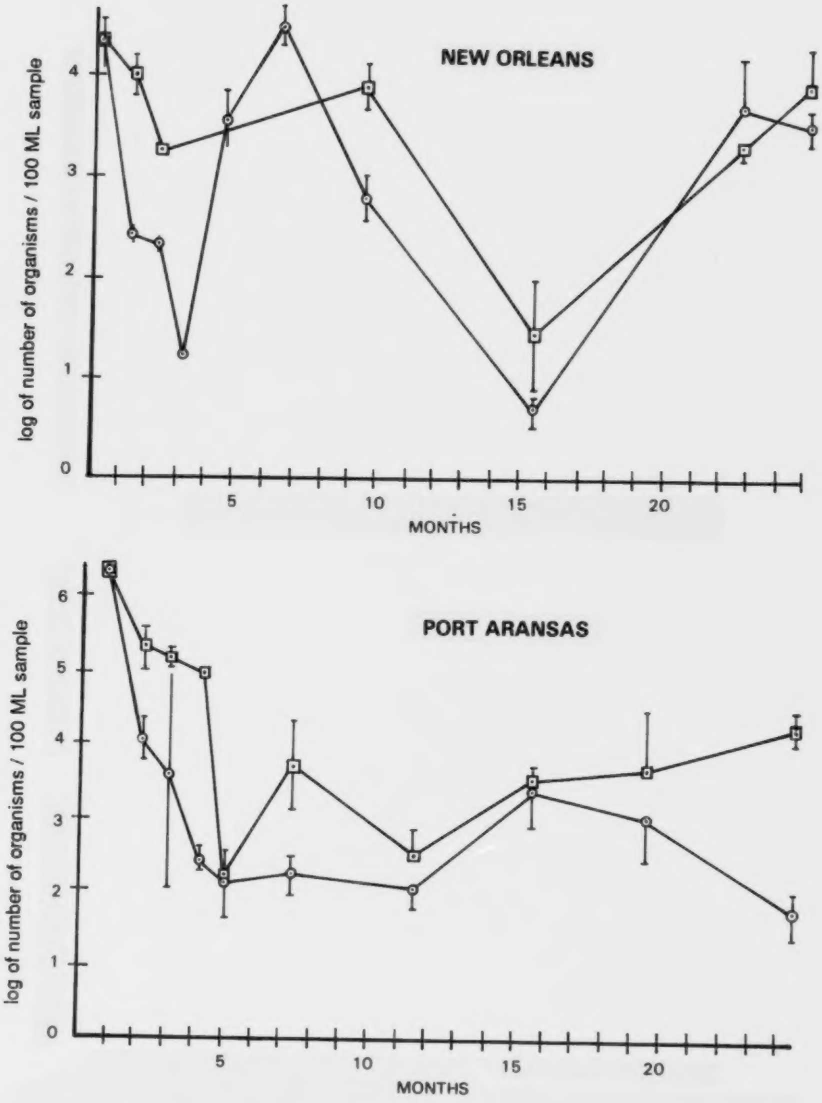

Figure 13. Graph of fecal streptococcal organisms per 100 milliliter versus time in months for New Orleans and Port Aransas sludge lagoons.

○ aqueous fraction
□ sediment fraction

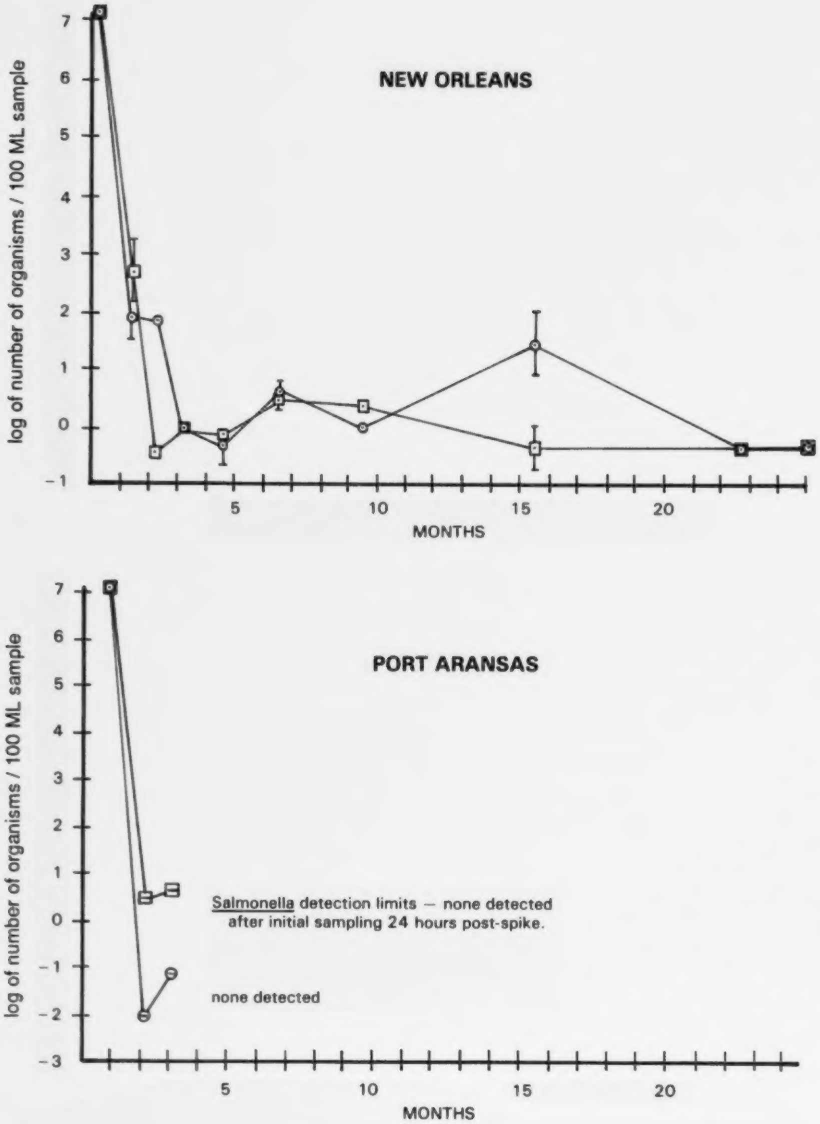

Figure 14. Graph of Salmonella organisms per 100 milliliter versus time in months for New Orleans and Port Aransas lagoons.

○ aqueous fraction
☐ sediment fraction

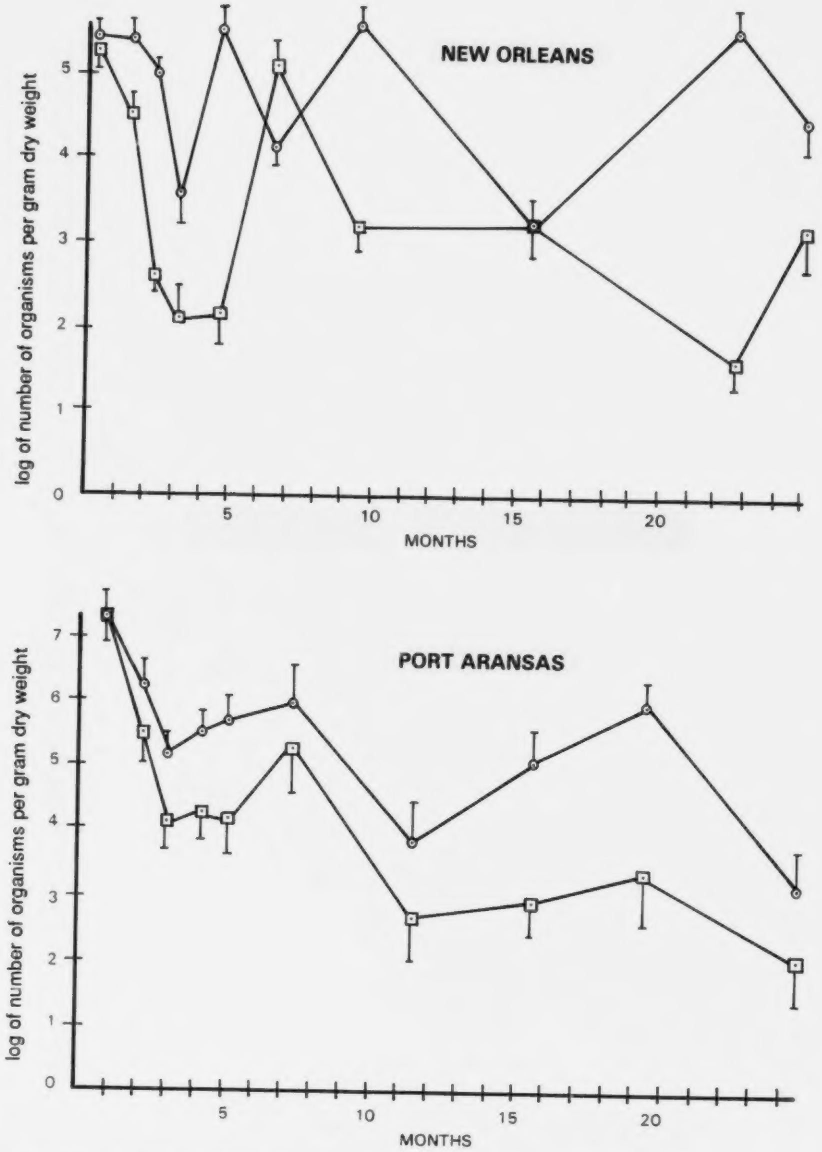

Figure 15. Graph of total coliform organisms per gram dry weight versus time in months for New Orleans and Port Aransas lagoons.

○ aqueous fraction
□ sediment fraction

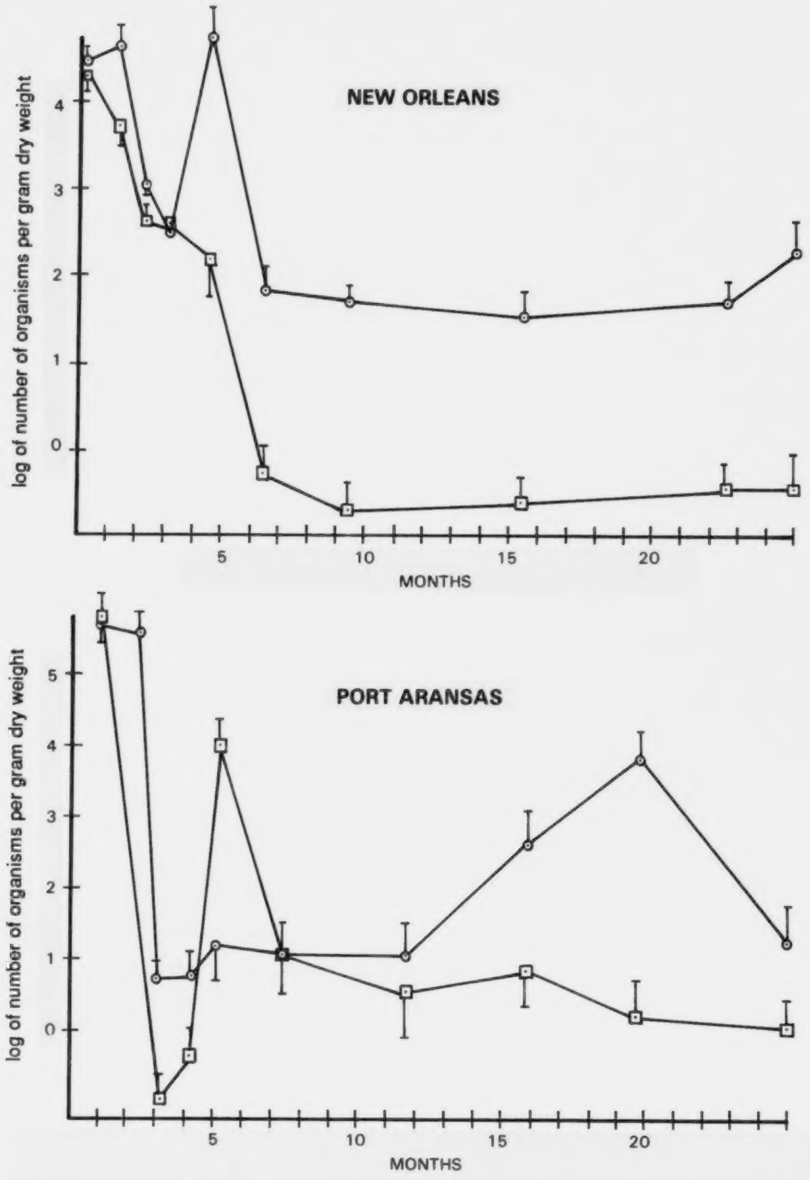

Figure 16. Graph of fecal coliform organisms per gram dry weight versus time in months for New Orleans and Port Aransas sludge lagoons.

○ aqueous fraction
□ sediment fraction

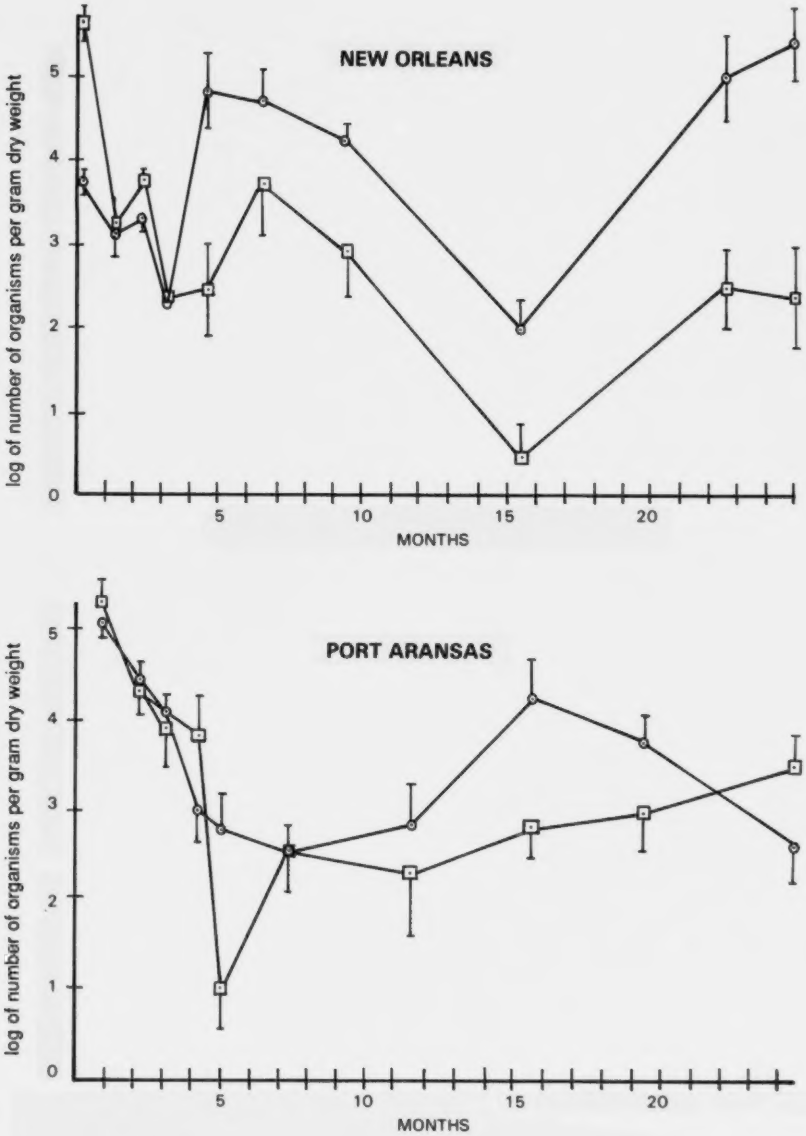

Figure 17. Graph of fecal streptococcal organisms per gram dry weight versus time in months for New Orleans and Port Aransas sludge lagoons.

○ aqueous fraction
□ sediment fraction

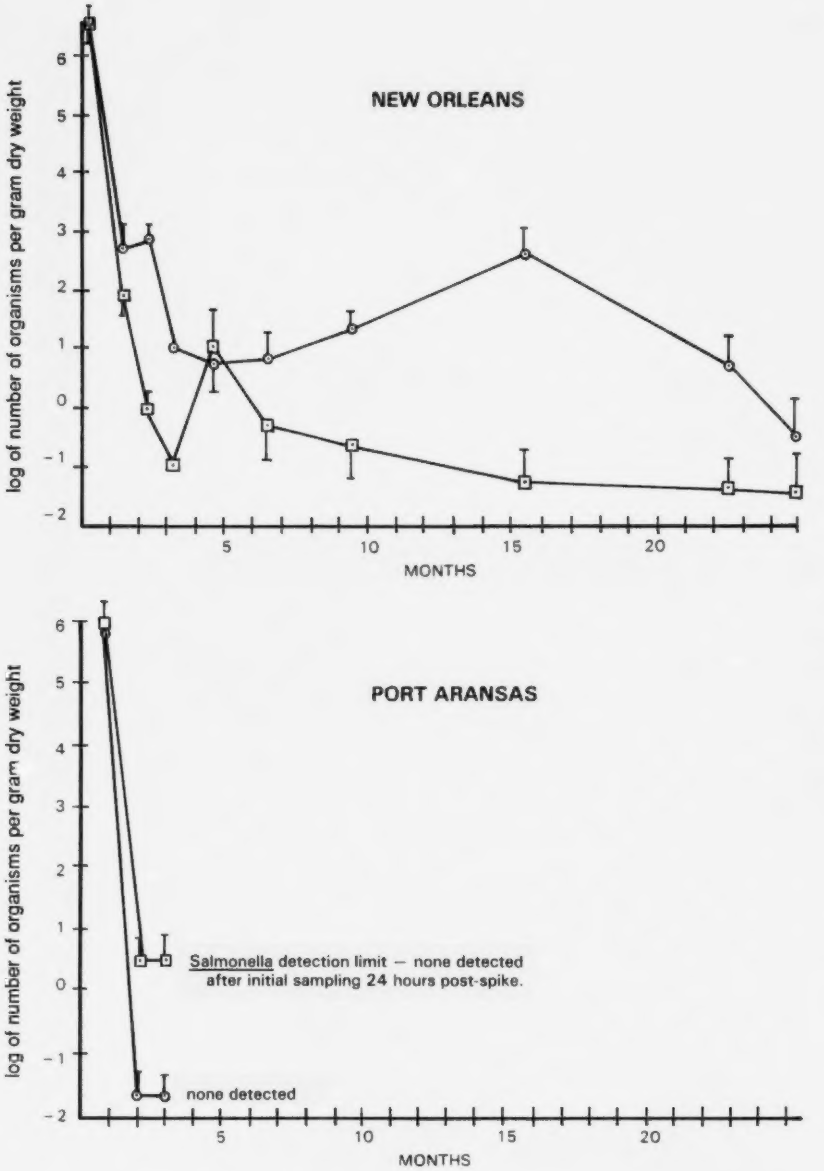

Figure 18. Graph of Salmonella organisms per gram dry weight versus time in months for New Orleans and Port Aransas sludge lagoons.

○ aqueous fraction
☐ sediment fraction

sites. Fecal coliforms died faster in both lagoons. Rates of 1.4 and 1.0 log of MPN's per 100 mL/month were observed respectively from sediments samples from New Orleans and Port Aransas lagoons. The Salmonella livingstone persisted for less than three months in both lagoons, and was not detected in the aqueous phase during the winter. The P values for fecal streptococcis regression analysis was not significant at both sites (Tables 19, 20, 21 and 22). Viruses and Salmonella were not detected within three months and could not be analyzed statistically. The data show all the trends in the reduction of pathogen respectively by volume and by dry weight in the New Orleans site and give the same information for the Port Aransas lagoon. The sludge characteristics varied significantly in both sites. The Port Aransas sludge, a combined municipal/industrial anaerobically digested sludge, had a lower pH and solid content, and a higher COD. After filling the lagoon, the solid fractions were actually floating after 24 hours. This sludge contained large quantities of oil, grit and grease, as noted by the percent volatiles and COD values. GC/MS analysis of the Corpus Christi sludge detected large amounts of petroleum refinery wastes. Chromatograms for the Port Aransas waste and a typical crude oil sample analysis showed that refractory petroleum residues, primarily the isoprenoids of 15 carbons or more in length, were the major constituents present in the Corpus Christi sludge, and responsible for its petroleum characteristics.

Another problem surfaced after four months. Initially it was determined that bacterial enumeration would be via the membrane filtration technique, since this method yields a direct count, while the multiple tube fermentation technique is based on a probability estimate. Due to the

TABLE 19: P VALUES, CORRELATION COEFFICIENTS AND INACTIVATION RATES (LOG OF MPN/100mL/MONTH) OF BACTERIA AND VIRUS IN NEW ORLEANS LAGOON (ON WET WEIGHT BASIS)

Parameters	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Salmonella	Virus
P Values	0.380[a]	0.011	0.730[a]	0.980[a]	0.32[a]
R	0.36	0.75	0.21	0.00	0.20
Rate (log of MPN/100mL/month)	0.30	1.4	0.04	0.83	-0.011

[a] Not significant

TABLE 20: P VALUES, CORRELATION COEFFICIENTS AND INACTIVATION RATES (LOG OF MPN/GM DRY WEIGHT/MONTH) OF BACTERIA AND VIRUS IN NEW ORLEANS LAGOON (ON DRY WEIGHT BASIS)

Parameters	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Salmonella	Virus
P Values	0.61[a]	0.05	0.63[a]	0.73[a]	NE[b]
R	0.26	0.62	0.25	0.20	NE
Rate (log of MPN/gm/month)	0.06	1.7	0.22	0.39	NE

[a] Not significant

[b] Not enough data to complete regression

TABLE 21: P VALUES, CORRELATION COEFFICIENTS AND INACTIVATION RATES (LOG OF MPN/100mL/MONTH) OF BACTERIA AND VIRUS IN PORT ARANSAS LAGOON (ON WET WEIGHT BASIS)

Parameters	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Salmonella	Virus
P Values	0.070[b]	0.013	0.960[a]	NE[c]	0.46[a]
R	0.59	0.74	0.00	NE	0.77
Rate (log of MPN/month)	0.72	0.99	0.013	NE	0.02

[a] Not significant

[b] Trend

[c] Not enough data to complete regression

TABLE 22: P VALUES, CORRELATION COEFFICIENTS AND INACTIVATION RATES (LOG OF MPN/GM DRY WEIGHT OF BACTERIA AND VIRUS IN PORT ARANSAS LAGOON (ON DRY WEIGHT BASIS)

Parameters	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Salmonella	Virus
P Values	0.003	0.96[a]	0.89[a]	0.034	NE[b]
R	0.82	0.08	0.13	0.99	NE
Rate (log of MPN/gm/month)	0.18	-0.21	-0.05	1.6	NE

[a] Not significant

[b] Not enough data to complete regression

nature of the sludge (oily grit in the Texas sludge and a large particulate matter in the Louisiana sludge) not remedied by homogenization, it was necessary to change the method of bacterial enumeration (raw and statistically analyzed bacterial data are listed in Appendix F). This was necessary because it was not possible to filter enough of the sample to get a bacterial count in the range of ten to one hundred per filter without clogging the membrane. The presence of solids on the membrane caused colony counts to vary significantly. Thus, the method of bacterial quantification was changed to MPN's per 100 mL.

Within two weeks an aqueous and sludge separation occurred in the New Orleans lagoon. The aqueous fraction began to lose anaerobic characteristics as noted by an increase in ORP values for the top fractions. The presence of a sludge blanket in the Port Aransas lagoon made this transition slower than observed in the New Orleans lagoon. After one month of storage, a two log drop in total and fecal coliforms and fecal streptococcal counts was observed in the aqueous phase of both lagoons. Similar reductions in sediment counts were also observed. Salmonella dropped from an initial concentration of 1×10^7 organisms per 100 mL to less than 2.5×10^2 organisms per 100 mL in the aqueous fractions of the New Orleans lagoon, and 2.5×10^3 organisms/100 mL in the sludge fractions. It is suspected that the consistently high temperature over nine months of the year at both locations were responsible for greater reduction in bacterial concentrations than noted in previous studies. It is common for temperatures to remain constantly above twenty degrees centigrade (even at night) for six months per year at both locations.

After two months there was < 3 organisms/100 mL of Salmonella in the New Orleans lagoon. Bacterial counts dropped an additional log during this period, and solids continued to concentrate at the bottom of the lagoon. With the beginning of spring came an increase in non-indigenous organisms present in the New Orleans lagoon. Mosquito larvae, spiders, and lizards began residing in the covered container, which had developed several small apertures, taking advantage of the shade and protection provided. No animals were seen in the Port Aransas lagoon except Black Widow spiders which built nests around the exterior.

After four months of storage, the aqueous fraction of both lagoons harbored large quantities of algae; i.e., unicellular green flagellates in the New Orleans lagoon and red algae in the Port Aransas lagoon. Their presence colored the collected top samples green or red, respectively, An odor of ammonia was noted above the surface waters of the New Orleans lagoon prior to sampling. Salmonella remained at a concentration of less than ten organisms per 100 mL in both aqueous and sediment fractions.

After ten months, total coliform and fecal streptococcal populations leveled off. The presence of intruder organisms (mostly insects) may have contributed to the total coliform counts in the New Orleans lagoon, although the Port Aransas lagoon showed similar counts without the presence of a large insect population. Fecal coliform levels continued to drop in both lagoons to less than 100 organisms/100 mL, with higher concentrations in the sediment fraction of the lagoon.

After a period of approximately 10 months, bacterial populations appeared to reach their minimum levels during the 24-month study. Fecal coliform levels were at a concentration of less than three organisms per 100 mL in aqueous and sediment phases of the lagoons, while total coliforms and fecal streptococcal organisms remained quite high. Total coliform levels returned to their initial high levels, due presumably to the presence of insect (larval stages) and animal (lizard and frog) inhabitants in and around the lagoon site. All bacteria monitored showed seasonal variations.

Results based on organisms per gram dry weight revealed that reductions in indicator bacterial were less than on a volumetric basis (per 100 mL), but still greater than 2-log and 4-log density reductions in both lagoon's total and fecal coliform bacterial counts. Fecal streptococcal bacterial counts were reduced in the sediment fraction of both lagoons, on a dry weight basis, a minimum of two logs in the Port Aransas lagoon with greater than three log reduction in the New Orleans lagoon. Both total coliforms and fecal streptococci reduction over two years were less than PFRP criteria and is conjectured to be the result of the influx of nutrients and other bacterial populations present in both lagoons after prolonged storage time. Salmonella livingstone (over six logs) reduction was down to below detection limits and water quality standards (<3 organisms/mL) in both lagoons.

The aqueous fraction of both lagoons evinced a lesser amount of bacterial reductions, possibly due to the presence of photosynthetic algae, which served as a source of nutrients. Since the aqueous fraction of the sludge lagoon is usually returned to the treatment plant, the higher bacterial counts present in the aqueous fraction were not considered to be a problem in lagoon storage.

VIRUS SURVIVAL

Poliovirus survived in the New Orleans lagoon's sludge sediment through 120 days, and was not detected at 180 days (Table G-1, Appendix G). In the liquid phase, it was detected at 60 days (Figures 19 and 20). In contrast, by day 120 Poliovirus survival in the Port Aransas lagoon was negligible in both the liquid and sediment fractions (Table G-2, Appendix G). However, low concentrations of virus were observed after 90 days of lagoon storage (Figure 21 and 22). Figure 23 shows the results from both sites. Converting the virus titration results from PFUs/gram dry weight did not significantly alter the relationship between virus survival in the liquid or sediment phases of the New Orleans lagoon. The same conversion when applied to Port Aransas titration results revealed a close relationship between virus survival in both the liquid and sediment phases (Figure 23).

The result of assaying the virus suspensions stored in vials placed in a sealed container deposited on the sediment of each lagoon revealed that in both lagoons the virus titers had decreased approximately 2 logs after 180 days. The effect of temperature on inactivation of the control virus suspensions was very similar for both lagoons.

The virus quality control and assurance data are presented in Table 23 for the New Orleans lagoon and Table 24 for the Port Aransas lagoon. The precision data is acceptable and almost identical for the split samples. The recoveries were higher at the New Orleans site (approximately 70-80 percent from the first to the thirtieth day with a drop to around 55 percent from 60 to 180 days). At the Port Aransas site, initial recoveries were around 60% percent dropping into the 40 percent range after the first day. The lower recoveries are probably a result of the high petroleum content at the Texas site.

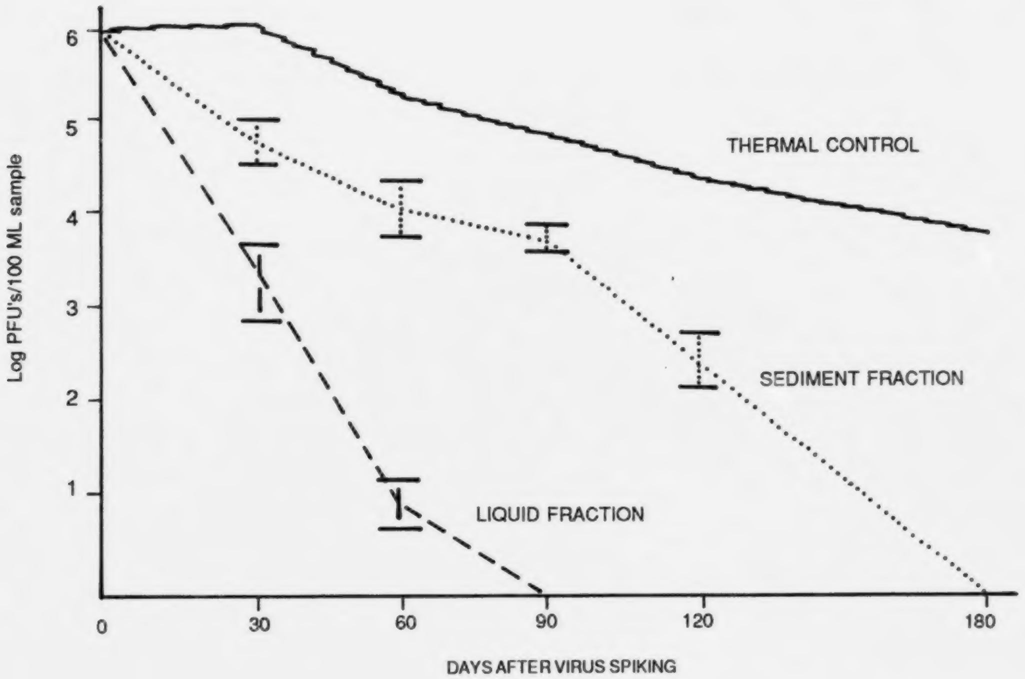

Figure 19. Poliovirus survival New Orleans lagoon.

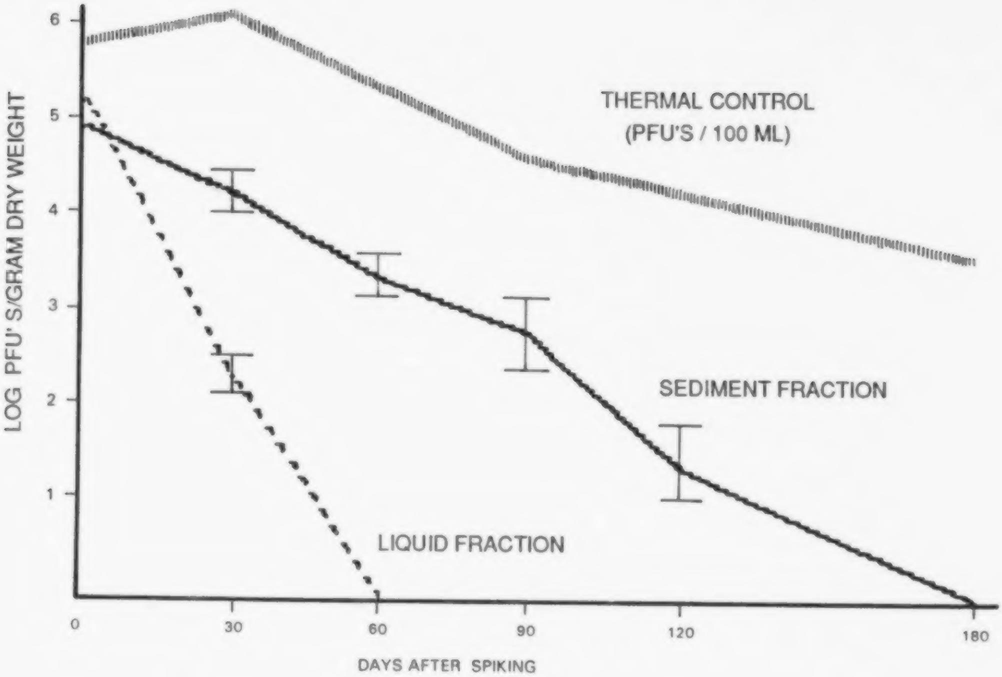

Figure 20. Poliovirus survival New Orleans lagoon (dry weight basis)

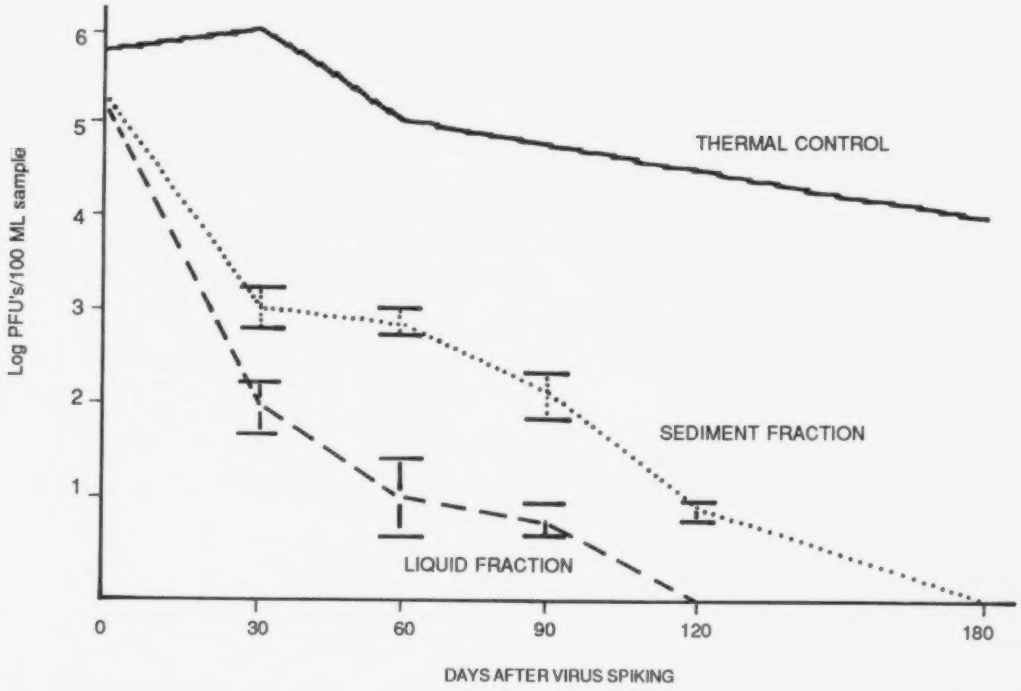

Figure 21. Poliovirus survival Port Aransas lagoon.

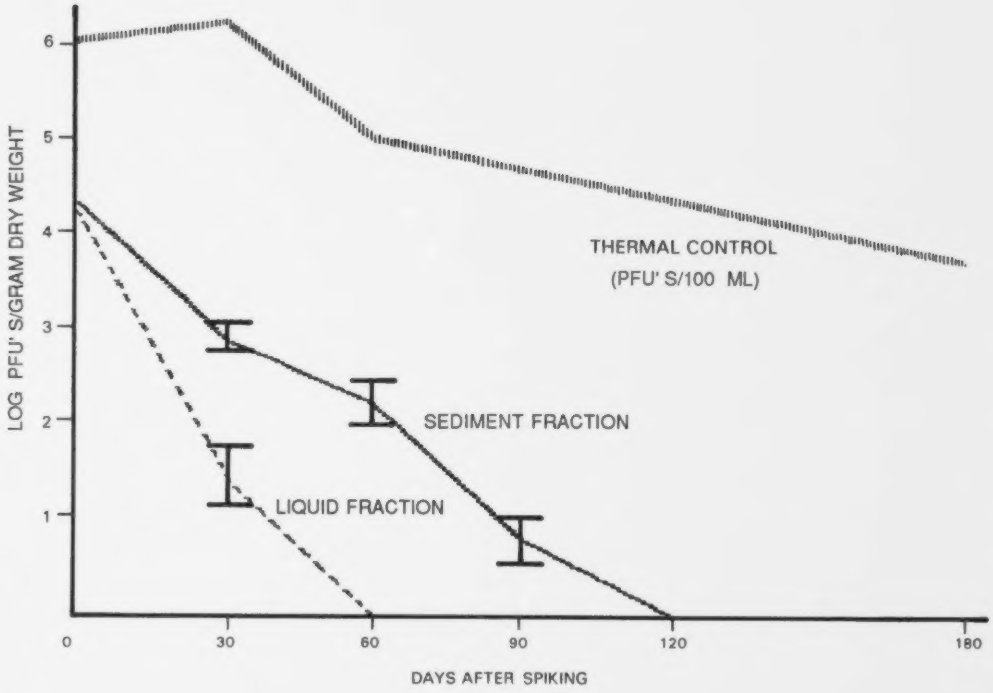

Figure 22. Poliovirus survival Port Aransas lagoon (dry weight basis).

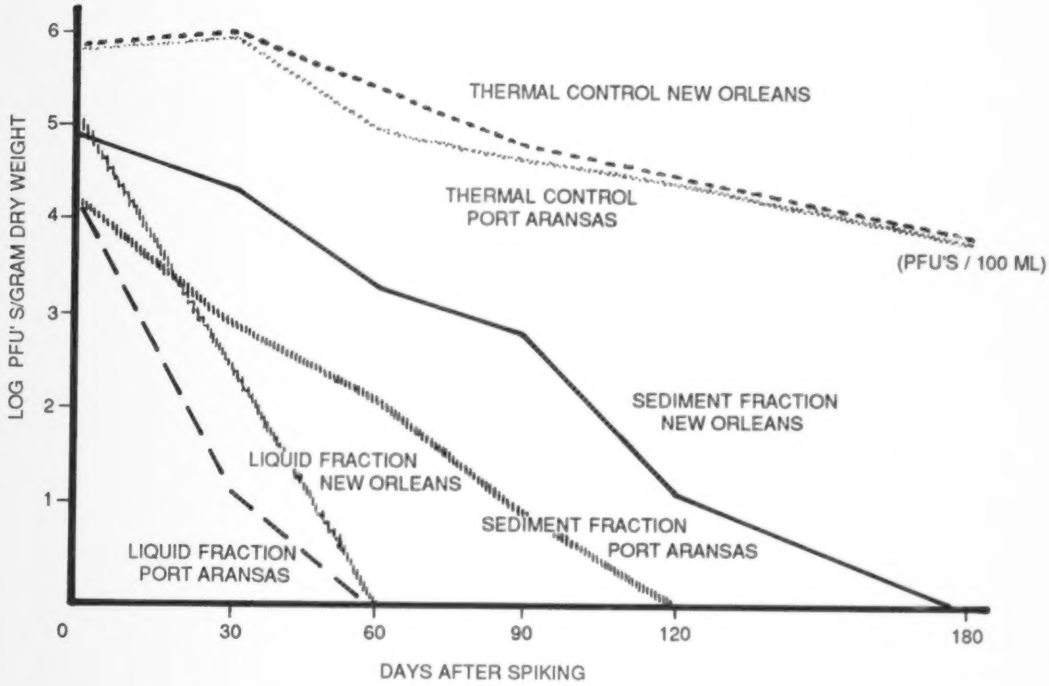

Figure 23. Composite of Poliovirus survival New Orleans and Port Aransas lagoons.

TABLE 23. SUMMARY OF QUALITY CONTROL DATA FOR POLIOVIRUS ANALYSIS: NEW ORLEANS LAGOON

Sample	PFU/100 mL Sludge					
	Day 1		Day 30		Day 60	
	Titer	% Recovery	Titer	% Recovery	Titer	% Recovery
5-1	7.5x10^{5}		1.1x10^{3}		--	
5-1A[a]	7.4x10^{5}		1.3x10^{3}		--	
5-1B[b]	1.4x10^{6}	80%[c]	7.3x10^{5}	73%[c]	5.5x10^{5}	55%[c]
5-2	8.4x10^{5}		7.5x10^{4}		1.2x10^{4}	
5-2A[a]	8.9x10^{5}		8.0x10^{4}		1.1x10^{4}	
5-2B[b]	1.6x10^{6}	85%[c]	8.6x10^{5}	79.5%[c]	6.9x10^{5}	69%[c]

Sample	PFU/100 mL Sludge					
	Day 90		Day 120		Day 180	
	Titer	% Recovery	Titer	% Recovery	Titer	% Recovery
5-1	--		--		--	--
5-1A[a]	--		--		--	--
5-1B[b]	6.2x10^{5}	62%[c]	--		--	--
5-2	6.3x10^{3}		2.8x10^{2}		--	--
5-2A[a]	6x10^{3}		2.9x10^{2}		--	--
5-2B[b]	5.8x10^{5}	58%[c]	6.4x10^{5}	65%[c]	5.5x10^{5}	55%[c]

[a] Duplicate titration for reproducibility

[b] Approximately 1x10^{6} PFU/100 mL of virus was added to 5-1B and 5-2 to determine recovery efficiency

[c] % Recovery = Obs. reading/1.0 x 10^{6} PFU's/100mL + Background Level x 100

TABLE 24. SUMMARY OF QUALITY CONTROL DATA FOR POLIOVIRUS ANALYSIS: PORT ARANSAS LAGOON

Sample	PFU/100 mL Sludge					
	Day 1		Day 30		Day 60	
	Titer	% Recovery	Titer	% Recovery	Titer	% Recovery
5-1	3.2×10^5		75		7.5	
5-1A[a]	3.1×10^5		98		--	
5-1B[b]	8.2×10^5	82%[c]	3×10^5	32.2%[c]	4.3×10^5	42.8%[c]
5-2	3.8×10^5		1.1×10^4		2.7×10^3	
5-2A[a]	4.2×10^5		1.1×10^4		2.9×10^3	
5-2B[b]	8.0×10^5	80%[c]	4×10^5	40.5%[c]	4.5×10^5	45.0%[c]

Sample	PFU/100 mL Sludge					
	Day 90		Day 120		Day 180	
	Titer	% Recovery	Titer	% Recovery	Titer	% Recovery
5-1	--		--		--	--
5-1A[a]	--		--		--	--
5-1B[b]	4.7×10^5	42.2%[c]	5×10^5	50.2%[c]	--	--
5-2	1.1×10^2		--		--	--
5-2A[a]	98		--		--	--
5-2B[b]	4.1×10^5	40.5%[c]	5.5×10^5	54.8%[c]	--	--

[a] Duplicate titration for reproducibility

[b] Approximately 1×10^6 PFU/100 mL of virus was added to 5-1B and 5-2 to determine recovery efficiency

[c] % Recovery = Obs. reading/1.0×10^6 PFU's/100mL + Background Level x 100

PARASITE SURVIVAL

Lagoon-Stored Sludge

The results of parasite analyses of samples from the two lagoons are illustrated in Figures 24 and 25 where the percent inactivation of *Ascaris* eggs over time is shown (the complete data are presented in Tables H-1 and H-2, Appendix H). In these figures the initial time (time 0) viability of the *Ascaris* eggs was calculated from the viability of the eggs in the spike rather than in the initial sample since the procedure used in examining the

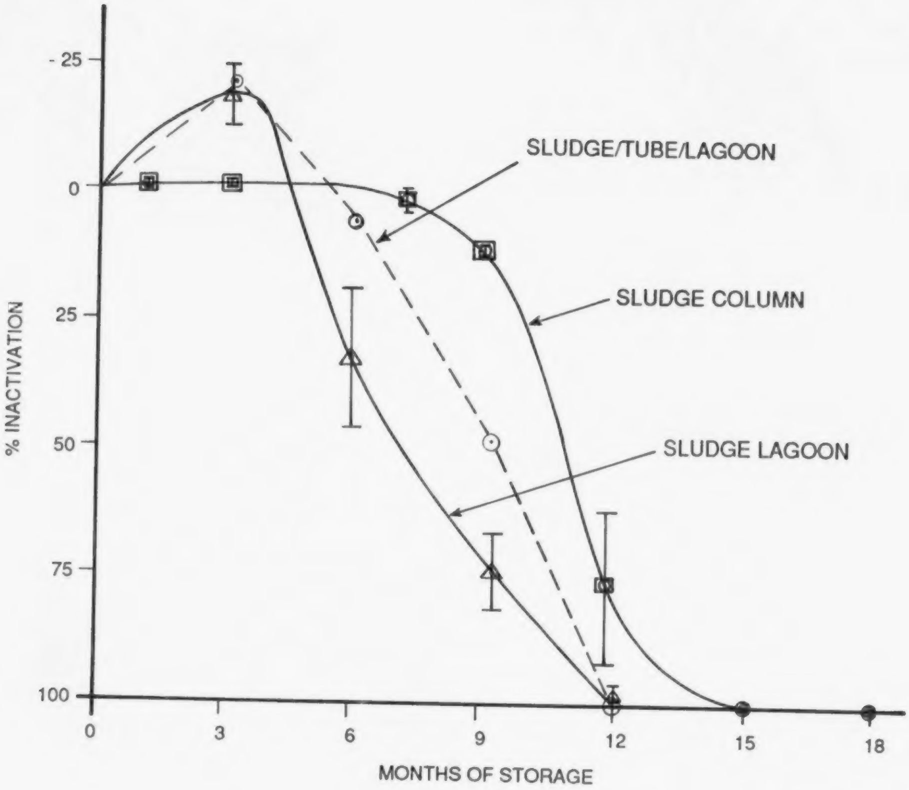

Figure 24. Inactivation of Ascaris eggs stored in New Orleans sludge in lagoon, tube in the lagoon, and large column.

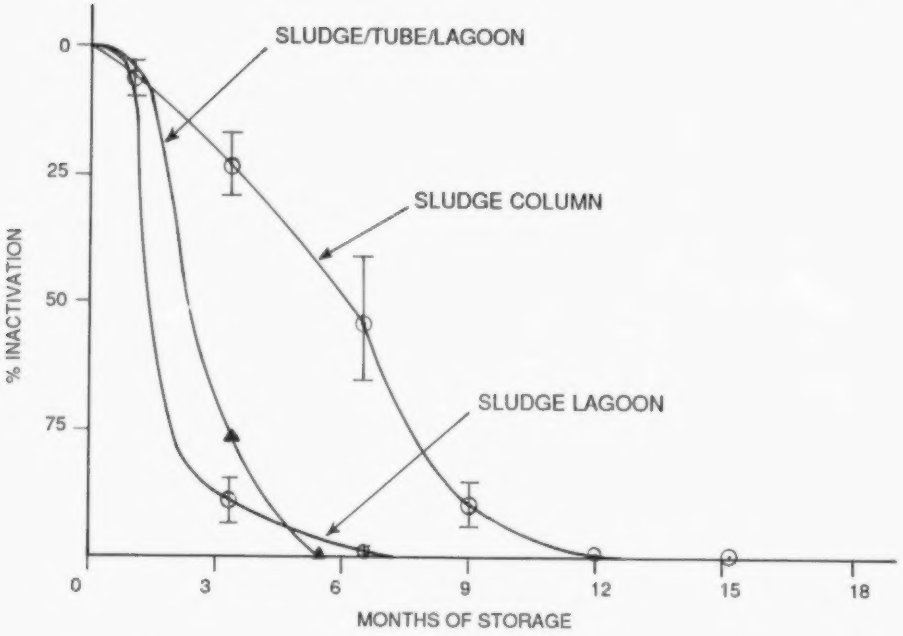

Figure 25. Inactivation of *Ascaris* eggs stored in Port Aransas sludge in lagoon, tube in the lagoon, and large column.

initial lagoon sample was found to have affected the ability of the eggs to develop in culture. As mentioned in Methods, a procedure that did not affect the viability of the eggs was used to examine all subsequent samples. The viability of the eggs in the spike added to the sludge in each lagoon was 13.7% (Tables H-1 to H-3, Appendix H). A concentration procedure was not used on the spike sample prior to the culturing of the eggs.

In the New Orleans lagoon, a die-off of the eggs began to occur after three months and by 6 months 33% of the viable eggs had been inactivated. By 9.3 months, 74% of the eggs had been inactivated, and by 12.2 months 98% of the eggs had been inactivated. No viable eggs were recovered subsequently.

In the case of the Port Aransas lagoon, the die-off of Ascaris eggs occurred much more rapidly (Figure 25). After three months 89% of the viable eggs in the original spike had been inactivated, and by 6.5 months nearly total inactivation (99.9% had occurred. The difference in the die-off of eggs in the two lagoons was significant (p=0.004).

Toxocara canis eggs were found to occur naturally in the sludges used to fill the two lagoons, but their density in each lagoon was very low. The presence and viability of these eggs were monitored in all samples. The data is presented in Table 25. They were found to die off fairly rapidly in each lagoon with those in the Port Aransas lagoon dying off earlier than those in the New Orleans lagoon. All eggs recovered after 3.4 months of storage in the Port Aransas lagoon were dead, while in the samples collected from the New Orleans lagoon a few eggs were still viable at 6 months. In samples collected initially and after three months of storage, the eggs in the New Orleans lagoon had a much higher rate of viability than those in the Port Aransas lagoon.

Control Samples

The small PVC tubes placed in each lagoon were filled with sludge spiked with a higher concentration of Ascaris eggs so that the die-off of eggs could be monitored more accurately. In the sludge in the tube placed in the New Orleans lagoon, it was observed that the eggs died off at about the same rate as the eggs in the lagoon sludge (Figure 24). At 9.3 months of storage, 49% of the viable eggs in the initial spike had been inactivated, and at 12.2 months and thereafter complete inactivation was observed. In the sludge in the tube placed in the Port Aransas lagoon, the rate of inactivation of the Ascaris eggs was similar to that of the eggs in the sludge in the same lagoon (Figure 25). The Ascaris eggs in distilled water in vials placed in each lagoon died off within about 6 months (Figure 26).

Sludge Stored in Large Columns

This part of the project was initiated to verify certain results obtained in the study of the survival of Ascaris eggs in sludge stored in the lagoons. These included the more rapid die-off of eggs in the sludge

TABLE 25. VIABILITY OF NATURALLY OCCURRING TOXOCARA EGGS RECOVERED FROM LAGOON-STORED SLUDGE[a]

NEW ORLEANS LAGOON	Months in storage								
Storage time	0	3.0	6.0	9.3	12.2	15.3	18.2	22.5	24.7
Total no. of eggs[b]	77	393	125	192	110	177	127	129	104
Total no. viable eggs[b]	53[c]	187[c]	2	0	0	0	0	0	0
Percent viability	68.8%	47.6%	1.6%	0%	0%	0%	0%	0%	0%
Total no. of samples	13	12	12	12	11	11	11	11	11

PORT ARANSAS LAGOON	Months in storage							
Storage time	0	3.4	6.5	10.6	14.7	18.8	23.7	28.5
Total no. of eggs[b]	359	224	149	100	48	82	63	27
Total no. viable eggs[b]	52[c]	2	0	0	0	0	0	0
Percent viability	15%	0.9%	0%	0%	0%	0%	0%	0%
Total no. of samples	12	12	12	11	12	12	11	7

[a] Viability of eggs determined by culturing.
[b] Total number of Toxocara eggs in all samples examined.
[c] Infective eggs fed to mice and larvae later recovered from liver.

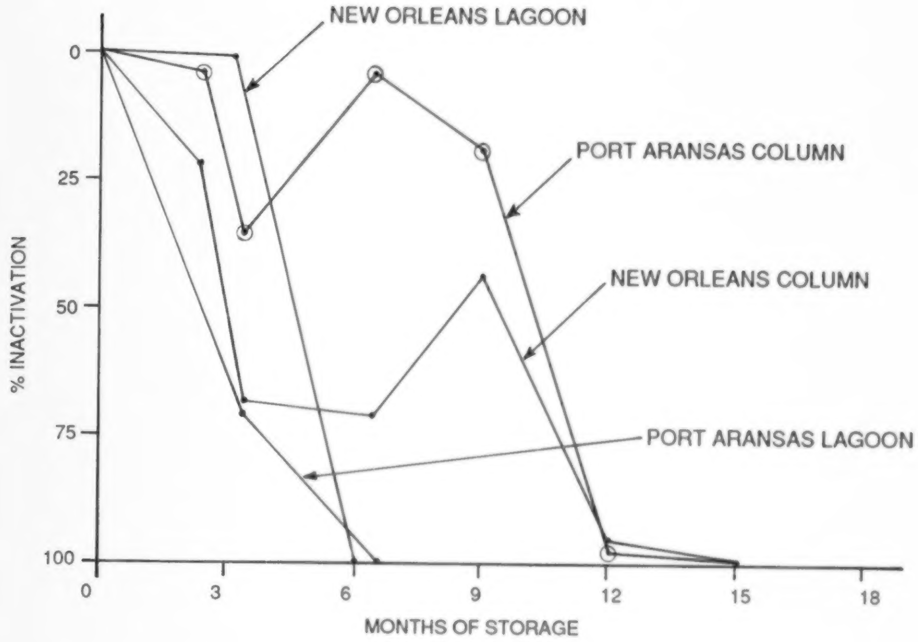

Figure 26. Inactivation of Ascaris eggs stored in distilled water in vials stored in New Orleans and Port Aransas lagoons and large columns (controls).

used in the Port Aransas lagoon as compared to the rate of die-off of eggs in the sludge in the New Orleans lagoon, and the rapid die-off of eggs in the distilled water controls in both lagoons. In addition, the *Ascaris* eggs used in spiking the sludge in each lagoon had a relatively low level of viability, 13.7%, and it was desirable to determine if another batch of *Ascaris* eggs with a higher initial rate of viability could survive longer in stored sludge.

The inactivation of *Ascaris* eggs in sludge stored in the two large plastic columns is shown in Figures 24 and 25 and Tables H-6 - H-9, Appendix H. The viability of the *Ascaris* eggs in the digested sludge used to spike these columns was 90.6%. In the column with New Orleans sludge, significant die-off did not occur until after 9 months of storage (Figure 24). At 9 months only 11% of the viable eggs in the initial samples had been inactivated, but after that the die-off was more rapid. At 12 months 76% of the eggs had been inactivated, and complete inactivation was observed at 15.2 months.

In the case of the eggs in the sludge of the Port Aransas column, i.e., sludge from Corpus Christi, Texas, the rate of inactivation of *Ascaris* eggs was less than that observed in the sludge in the Port Aransas lagoon but was greater than that observed in the New Orleans column (Figures 24 and 25). In the Port Aransas column, the inactivation of the eggs occurred at a fairly steady rate with 7% inactivation observed at one month, 23% at 3.3 months, 54% at 6.4 months, 90% at 9 months and nearly complete inactivation (99.9%) at 12 months. Although the time it took for complete inactivation of eggs to occur in the sludge in the two columns was not statistically different, the inactivation that had occurred in the Port Aransas column at 6 months and 9 months was significantly greater than the inactivation that had occurred at these times in the New Orleans column ($p < 0.05$).

Quality Control

The survival of eggs in the distilled water controls placed in the two columns was similar ($p = 0.42$) (Figure 26 and Table H-10, Appendix H), although there was considerable fluctuation in the viability of the eggs from individual vials examined between three and 12 months of storage. In the vials examined from each column at 12 months, nearly all of the eggs had died (96% and 98%), and at 15.2 months no viable eggs were observed in vials taken from the two columns. In Figure 27 the inactivation of eggs in these distilled water controls are compared to the inactivation of eggs in the column with the New Orleans sludge.

The quality control data are summarized in Tables 26 and 27; the complete data are shown in Tables H-5 and H-12, Appendix H. The precision of the parasitologic technique was 14.8% for all the samples examined (Table 26). The precision for sludge samples from different sources varied from a mean of 10.0% to 19.0%. The accuracy of the technique for all samples examined was 75.5%, and again it varied for different sludges (Table 27), i.e., 62.9 to 82.1. The quality control results were within acceptable limits.

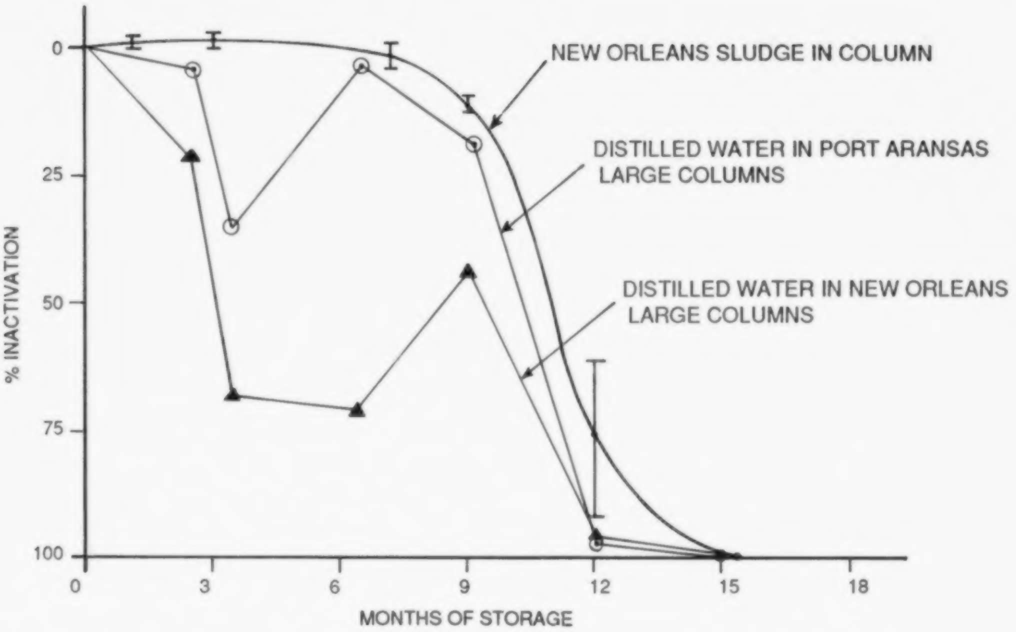

Figure 27. Inactivation of Ascaris eggs in New Orleans sludge in large column, and in distilled water in vials placed in New Orleans and Port Aransas columns.

TABLE 26. SUMMARY OF QUALITY CONTROL DATA FOR PARASITOLOGIC ANALYSIS OF LAGOON-STORED SLUDGE FOR *ASCARIS* EGGS.
A. PRECISION

Sample type	Number of analyses	Mean Variability[a] (± S.D.)
New Orleans lagoon	11	19.0% (±16.0%)
Port Aransas lagoon	8	14.4% (± 8.7%)
New Orleans sludge in large column	7	10.0% (± 6.5%)
Port Aransas sludge in large column	7	13.4% (±12.5%)
All Samples	33	14.8% (±12.5%)

[a]

$$\frac{\text{Sum}\ \dfrac{\text{Standard deviation of no. of eggs recovered from duplicate samples}}{\text{Mean no. of eggs recovered from duplicate samples}} \times 100}{\text{Number of precision analyses}}$$

TABLE 27. SUMMARY OF QUALITY CONTROL DATA FOR PARASITOLOGIC ANALYSIS OF LAGOON-STORED SLUDGE FOR *ASCARIS* EGGS.
B. ACCURACY

Sample type	Number of analyses	Mean percent recovery of eggs from spiked samples (± S.D.)
New Orleans lagoon	9	82.1% (±17.3%)
Port Aransas lagoon	7	62.9% (±11.0%)
New Orleans sludge in large column	6	81.8% (±37.0%)
Port Aransas sludge in large column	7	74.4% (±23.0%)
All Samples	29	75.5% (±24.4%)

Infectivity of Ascaris Eggs

The infectivity of cultured Ascaris eggs is shown in Table H-13, Appendix H. In most cases, the recovery of larvae from the lungs of mice was lower when the "infective" eggs from sludge or control samples were fed to mice than when "infective" eggs from control cultures were fed to mice. However, in a few cases the opposite was true. This procedure, as well as other procedures used for testing the infectivity of eggs in animals, is imprecise, and the results reflect that fact. However, the results do indicate that mice could be infected with eggs recovered from sludges after varying periods of time. They also indicate that although, in some cases, larvae within the egg shell were motile and appeared to be infective, they would not infect mice.

Sludge Settling Experiment

The results of settling experiment are shown in Table H-14 - H-15, Appendix H. When the anaerobic sludge containing Ascaris eggs was allowed to settle under quiescent conditions, some eggs still remained in the upper foot of the sludge for at least seven days. However, at twenty-four hours only approximately 20% of the original number of eggs remained in the upper one-half (one meter) of the settled sludge and, after one week, less than 1% of the eggs were in the upper one-half of the sludge. After one month, all of the eggs were found in the bottom three feet of the sludge column. The solids in the sludge settled at about the same rate as the Ascaris eggs.

STATISTICAL ANALYSIS OF ABIOTIC PARAMETERS

The influence of the various abiotic parameters on the inactivation of the selected pathogens are illustrated in Tables 28, 29 and 30. Table 30 presents the relationship of the bacterial indicators with respect to their aqueous phase and respective sludge and their counterpart at the other site.

In assessing inactivation of Poliovirus Type 1 in the supernatent fraction, no significance for any of the parameters at New Orleans was detected, but significance was noted with respect to time, conductivity and ammonia for the Port Aransas lagoon. Salmonella die-off showed no significance for these same parameters at the Port Aransas site but showed significance with respect to oxidation-reduction potential, total solids, volatile solids and chemical oxygen demand at New Orleans. Ascaris viability was not related to any of these abiotic parameters at either lagoon. The fecal coliform decrease was significant with respect to time, oxidation-reduction potential and conductivity for the New Orleans lagoon. In the Port Aransas lagoon, the inactivation of fecal coliforms was related to total solids, volatile solids and chemical oxygen demand. Total coliform inactivation was significantly related to ammonia, time and pH in the Port Aransas lagoon, but not correlated to any parameter in the New Orleans lagoon.

TABLE 28. SIGNIFICANCE OF ABIOTICS ON THE INACTIVATION OF PATHOGENS IN THE AQUEOUS SAMPLES WITH RESPECT TO PROBABILITY (SIGNIFICANT - SP<0.05; TREND - (T) 0.05<P<0.1; NOT SIGNIFICANT (N.S.) - P>0.1)

Pathogens	Poliovirus Type 1		*Salmonella* sp.		Total Coliform		Fecal Coliform		Fecal *Streptococci*		*Ascaris* Eggs	
Parameter	P[a]	N[b]	P	N	P	N	P	N	P	N	P	N
Time (days)	<0.1	NS	NS	NS	<0.05	NS	NS	<0.05	NS	NS	NS	NS
pH (S.U.)	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	NS	NS	NS
Temperature (°C)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Oxidation Reduction Potential (orp-mv)	NS	NS	NS	<0.025	NS	NS	NS	<0.05	NS	NS	NS	NS
Conductivity (umhos/cm)	<0.1	NS	NS	NS	NS	NS	NS	<0.01	NS	NS	NS	NS
Total Solids (mg/L)	NS	NS	NS	<0.001	<0.1	NS	<0.1	NS	<0.05	NS	NS	NS
Volatile Solids (mg/L)	NS	NS	NS	<0.001	<0.1	NS	<0.1	NS	<0.1	NS	NS	NS
Chemical Oxygen Demand (COD-mg/L)	NS	NS	NS	<0.001	<0.1	NS	<0.1	NS	<0.1	NS	NS	NS
Ammonia (NH_3) (mg of N/L)	<0.01	NS	NS	NS	<0.025	NS	NS	NS	NS	NS	NS	NS

[a] Port Aransas [b] New Orleans

TABLE 29. SIGNIFICANCE OF ABIOTICS ON THE INACTIVATION OF PATHOGENS IN SEDIMENT WITH RESPECT TO PROBABILITY (SIGNIFICANT - $P<0.05$; TREND (T) - $0.05<P<0.1$; NOT SIGNIFICANT (N.S.) - $P>0.1$)

Pathogens	Poliovirus Type 1		*Salmonella* sp.		Total Coliform		Fecal Coliform		Fecal *Streptococci*		*Ascaris* Eggs	
Parameter	P[a]	N[b]	P	N	P	N	P	N	P	N	P	N
Time (days)	<0.025	<0.05	NS	NS	<0.01	NS	<0.1	<0.01	NS	NS	NS	NS
pH (S.U.)	<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature (°C)	<0.1	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	NS	NS
Oxidation Reduction Potential (orp-mv)	NS	NS	NS	NS	<0.1	NS	NS	<0.02	NS	NS	NS	NS
Conductivity (umhos/cm)	NS	NS	NS	NS	NS	NS	NS	NS	<0.025	NS	NS	NS
Total Solids (mg/L)	<0.025	NS	NS	NS	NS	NS	NS	NS	<0.1	<0.01	NS	<0.1
Volatile solids (mg/L)	<0.05	NS	NS	NS	NS	NS	NS	NS	<0.1	<0.01	NS	<0.005
Chemical Oxygen Demand (COD-mg/L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ammonia (NH_3) (mg of N/L)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Port Aransas lagoon [b] New Orleans lagoon

TABLE 30. CORRELATION OF INDICATOR BACTERIAL DATA TO RESPECTIVE SEDIMENT AND AQUEOUS SAMPLES

Lagoon Site	Phase Sampled	Specific Pathogenic Indicator	Potential Correlation With Below Sediment or Above Aqueous Fractions	Potential Correlation With Other Site Aqueous and Respective Pathogen
Port Aransas	Aqueous	Total Coli.	0.00	0.79[a]
	"	Fecal Coli.	0.00	0.08
	"	Fecal Strep.	0.00	0.39
	Sediment	Total Coli.	0.00	0.003
	"	Fecal Coli.	0.00	0.00
	"	Fecal Strep.	0.00	0.00
New Orleans	Aqueous	Total Coli.	0.87[a]	0.79[a]
	"	Fecal Coli.	0.24	0.08
	"	Fecal Strep.	0.36	0.39
	Sediment	Total Coli.	0.87[a]	0.003
	"	Fecal Coli.	0.24	0.00
	"	Fecal Strep.	0.36	0.00

[a] Potentially close to probability of 0.9 or close to a statistical trend.

With regards to the sediment fraction, Poliovirus Type 1 inactivation was significant for time, pH, temperature, total solids, and volatile solids at Port Aransas, while only time was noted to be significant at New Orleans. No significant abiotic parameter was found to be significant for Salmonella inactivation at either lagoon. Ascaris egg inactivation was not related to any parameter in the Port Aransas lagoon and was related only to volatile solids in the New Orleans lagoon. In looking at the indicator bacterial groups, total coliform inactivation was not related to any parameter at the New Orleans lagoon, but influence was reported for time and oxidation-reduction potential at the Port Aransas site. Similarly, fecal coliform inactivation was significant only for time at Port Aransas. Yet at the New Orleans site, fecal coliform inactivation was related to time and oxidation-reduction potential. Finally, fecal streptococci inactivation was significant with respect temperature, total solids and volatile solids in the New Orleans lagoon, but in the Port Aransas lagoon was significantly correlated to only conductivity.

Table 30 indicates that there was no significant correlation between the aqueous and sediment fractions and any of the bacterial indicators at either site.

SECTION 7

DISCUSSION

The discussion is presented in four parts: bacterial, viral, parasite survival, and statistical analysis. The first three parts discuss the survival of the specific groups of microbes with respect to lagoon storage and the possible influence of petroleum residues. The statistical section discusses the correlation of all biotic and abiotic factors with respect to microbial inactivation.

BACTERIAL SURVIVAL

The data pertaining to the inactivation of indicator bacteria indicated that the survival patterns for the coliforms and fecal streptococci were of the order of 2 to 4 magnitudes of inactivation over a time period of one year. Accordingly, this would mean that the use of lagoon storage for inactivating bacteria contained in municipal sludges is feasible in such semi-tropical regions as the southern parts of Louisiana and Texas.

The declines in indicator bacteria (log/MPN/gm suspended solids) was preceded by a significant decrease in Salmonella during the first three months of lagoon storage. However, the correlation of decreases in indicator bacteria with decreases in Salmonella appeared to be nebulous. This becomes more obvious when one examines the increases in indicator organisms concentrations after the first year of storage which continued through the second year as a possible result of outside contamination. Examining Figures 11 through 13 and Figure 15 through 17 and comparing with Figures 14 and 18 confirms the lack of synchrony between Salmonella and indicator bacteria survival during lagoon storage under semi-tropical conditions. This finding is in contrast to the report of Stern and Ferrell (16) in which the authors indicated that Salmonella sp. survival was similar to indicator bacteria following lagoon storage of anaerobically digested sludge at 20°C or more. It was also conjectured that reduction appeared to be a function of temperature, sludge composition, storage time, and type of sludge stabilization, and such variables might account for the differences noted in the report presented herein. For instance, when decay rate coefficients were determined, the Salmonella inactivation was greater than the indicator bacteria by a rate of 1.2 logs of MPN/100 mL/month. The Salmonella results were similar to the report of Pedersen (5) who reported a three log reduction in pathogenic bacteria following lagoon storage for four months at 20°C. The importance of the reduction of Salmonella by more than 5 logs observed in the study reported on herein means that lagoon storage under certain semi-tropical conditions meets the criteria for PFRP in relation to inactivation of pathogenic bacteria.

VIRUS SURVIVAL

As can be seen in Figure 23, the analyses results for thermal control samples from both the New Orleans and the Port Aransas lagoons revealed nearly identical decreases in virus concentrations after 180 days of storage in the lagoons' sediment fraction. While the thermal control concentrations decreased by approximately two logs, in contrast, the liquid and sediment fraction samples collected over the same time period evinced concentration reductions approaching 6 logs.

For both lagoons, Poliovirus survival was significantly greater in the sediment than the liquid fractions. Initially it was suspected that survival in the liquid phase was greater in the New Orleans than the Port Aransas lagoon but dry weight conversions showed that the survival pattern differences could be attributed to differences in initial concentrations following spiking. As can be seen in Figure 23, which compares dry weight titration results for both the New Orleans and Port Aransas lagoon virus samples, if adjustments are made for the differences in initial spiking concentrations (approximately one log), the inactivation curves over storage time would be nearly identical. Thus the titration differences could be attributed to two facts: (1) the initially lower virus concentrations in the Port Aransas lagoon could have been from poor mixing and sampling, or (2) the petroleum constituents in the Port Aransas Lagoon sludges reacted immediately to inactivate the spiked virus during mixing but after mixing and lagoon storage the chemical constituents had little or no effect on virus survival.

Comparison of the results from this study with previously published reports is handicapped because of the spiking concentrations of Poliovirus introduced in both the New Orleans and Port Aransas lagoons. Most other reports observed indigenous enterovirus inactivation with a greater than 1 log reduction after two months at 29°C (16), complete inactivation of detectable viruses within 2 months following storage under semi-tropical conditions (37), and complete inactivation of detectable enteroviruses within 2 months (38). It is thus apparent that the use of lagoon storage of municipal sludges under nearly identical semi-tropical climatic conditions will result in the inactivation of enteroviruses after 6 months.

PARASITE SURVIVAL

The storage of Ascaris eggs in sludges in lagoons and in large columns produced several interesting results. First of all, the eggs survived for a significantly shorter time in the Port Aransas lagoon than in the New Orleans lagoon (Figures 24 and 25). In the Port Aransas lagoon, 89% of the Ascaris eggs had died by 3.4 months of storage, and essentially all (99.9%) had died by 6.5 months. However, in the New Orleans lagoon, only 32.5% of the eggs had died by 6 months. At 9.3 months, 74% of the eggs had been inactivated, and it was 12.2 months before nearly complete inactivation had occurred. The difference in the survival of eggs in the two lagoons was probably due to the presence of petroleum by-products in the sludge in the Port Aransas lagoon which affected the viability of the eggs. Since the temperatures observed in the two lagoons varied very little from each other, temperature was unlikely to have caused the observed difference in *Ascaris* egg inactivation.

Due to the differences in the survival of eggs in the sludges in the two lagoons, a second experiment was initiated in which *Ascaris* eggs were stored in sludge from New Orleans and in sludge from Corpus Christi, Texas, the source of the sludge used in the lagoon experiment. In this experiment, the spiked sludges were stored in large plastic columns (392 L of sludge in each) rather than in lagoon boxes, and both columns were placed next to each other in New Orleans. Another reason for initiating this study was that the viability of the *Ascaris* eggs used to spike the lagoon-stored sludges was relatively low, 13.7%, and it was desirable to do an experiment where *Ascaris* eggs with a higher initial rate of viability were used to spike the sludges. The low percentage of viable eggs in the spike used in the lagoon experiment was primarily due to the procedures used to collect the eggs and the fact that this was the first attempt to collect such a large number of *Ascaris* eggs. The eggs for the lagoon experiment were collected in the field from the feces of naturally infected pigs near Lubbock, Texas, during the summer of 1984. There was some storage of the feces diluted in water under very warm conditions in the field before the concentration procedures could be done in the laboratory. The collection of the eggs to spike the sludges placed in the two columns was carried out in a much cooler time of the year, and the procedures were improved by reducing the time required to concentrate the eggs prior to storing them under refrigeration. Consequently, the eggs used to spike the sludges in the columns had a relatively high rate of viability, 90.6%. In the case of New Orleans sludge in the column, little inactivation of eggs occurred for the first 9 months of storage (11% inactivation at 9 months), while in the lagoon, 74% of the eggs had been inactivated at 9 months (Figure 24). This difference was significant. At 9 months, the viability of the eggs in both the column and the lagoon had dropped to low levels, i.e., 76% inactivated in the column and 98% in the lagoon. These differences were not significant. In the case of the Port Aransas (Corpus Christi) sludges, the eggs survived significantly longer in the column than in the lagoon (Figure 25). At 3.4 months of storage, 89% of the eggs in the lagoon had been inactivated while only 23% of the eggs in the column had been inactivated at 3.3 months. At 6.5 months, there was nearly complete inactivation of eggs in the lagoon (99.9%), but in the column only 54% of the eggs had been inactivated. This difference was also significant. Complete inactivation of eggs in the Port Aransas column was not observed until 12 months of storage. It is concluded that the higher initial viability of the eggs used to spike the sludges in the two columns was mainly responsible for the longer survival of the eggs. Since the temperatures recorded in the columns and the lagoons were very similar, it seems unlikely that the differences observed were due to temperature effects. Actually, the storage of sludge in the two columns was initiated in July 1985 when the temperature of the sludge in each column was 26°C, and this temperature persisted for at least three months. On the other hand, the storage of sludge in the two lagoons was initiated in February 1984 when the temperatures in the New Orleans and Port Aransas lagoons were 14°C and 19°C, respectively. Since the eggs in the columns were initially exposed to higher temperatures than those in the lagoons, they should have been affected more by the initial temperatures. The fact that they survived longer supports the idea that the shorter survival of eggs in the lagoons was not due to temperature effects.

The sludge temperatures in the two lagoons fluctuated to higher and lower levels than they did in the two columns. Two possible reasons for this is that the surface area of each lagoon was relatively large (3.2 square meters) while that of the columns was small (0.07 square meter), and the top of the lagoon was covered only by a screen while the top of the column was capped. The sludge in the lagoon was thus more likely to have been affected by air temperature and sunlight than the sludge in the columns.

In all but one occasion, the temperature readings of the top and bottom portions of the sludge in the lagoons were within one degree of each other, but on 14 November 1984 the reading in the upper level of the New Orleans lagoon was 18°C, and the temperature in the bottom was 14°C.

While the survival of Ascaris eggs in the New Orleans and Port Aransas columns was not significantly different in regard to the time it took for complete inactivation, the rate of inactivation in the early months of storage was different. In the New Orleans column, little inactivation had occurred by 9 months (11%), but in the Port Aransas column 90% of the eggs were inactivated at 9 months. This difference may have also been due to the presence of petroleum by-products in the Port Aransas sludge since the temperature in the two columns did not differ.

The finding that the Ascaris eggs in the distilled water control samples placed in the lagoons survived for a shorter time than did the eggs in the stored sludge in the two lagoons was unexpected (Figure 26). In preparing these control samples, 1 ppm of sodium sulfite was added to the distilled water placed with the eggs in the vials to scavenge any oxygen that might be present so that the environment in the vials would simulate the environment in the lagoon-stored sludge. When the control tubes examined at six months and thereafter were opened, a noticeable odor of hydrogen sulfide was present. On the possibility that the sodium sulfite might have affected the Ascaris eggs in these control vials, a different procedure was followed when the sludge storage experiment was repeated and spiked sludge was stored in large plastic columns rather than in lagoons. The distilled water control samples placed in the sludges stored in these two columns contained eggs in deoxygenated water to which nothing had been added. In these control samples 50% or more of the eggs survived for nine months, but in the samples examined after 12 months the number of viable eggs present was greatly reduced (Figure 26). No viable eggs were found in these control samples after 12 months. Although there was some fluctuation in the viability of eggs examined from individual vials from the two columns between 3 and 9 months of storage, the survival of the eggs appears to be essentially the same as the eggs in the sludge in the New Orleans column (Figure 27).

Since no other study of this type had been attempted, there was no information available for guidance on how Ascaris eggs serving as controls should be stored in these circumstances. For experimental studies that require infective eggs of Ascaris, investigators generally culture the eggs in an aqueous medium with a chemical added to prevent the growth of bacteria or fungi. Dilute acids, alkalies or formaldehyde have been used for such purposes. Reports on the maximum longevity of eggs in such media are lacking. It was decided not to use a chemical in the distilled water to prevent the growth of micro-organisms in the vials since the long term effects of these chemicals on Ascaris eggs has not been documented. Also, the eggs were not treated with a chemical to disinfect the surface since disinfectants commonly used, such as sodium hypochlorite and hydrogen peroxide, are known to affect the outer shell of the eggs. The purpose of these controls was not to provide a medium for the eggs to develop the infective stage, but to simulate the anaerobic conditions of the lagooned sludge without the presence of all of the elements normally found in sludge. It is possible that bacteria or fungi could have been on the surface of the eggshells and subsequently grew during the storage of the eggs and directly affected the viability of the eggs. Although the same micro-organisms might have been introduced with the eggs into the stored sludge, in the sludge they would have had a nutrient source other than the eggs, while in the distilled water control, the eggs would have been the only source of nutrients. Little is known about the requirements for the long term storage under anaerobic conditions. Kmetec et al. (46) stored eggs in 0.1 N sulfuric acid and found that after 16 weeks there was a 25% loss of viability of the eggs. They did not report on observations over a longer period.

There are no other studies on the survival of Ascaris eggs in lagoons to which the results of the present study can be compared. O'Donnell, et al. (1) studied the survival of various helminth eggs including Ascaris suum eggs after storage of small samples (100 g wet weight) at constant temperatures, 4°C and 25°C, or in containers buried in the ground near Cincinnati, Ohio. The results they observed in regard to the survival of Ascaris eggs are worth examining. At three months of storage and thereafter, they recovered a low (15% to 25%) to very low (less than 10%) percentage of the Ascaris eggs that had been added initially to the samples. The samples included aerobically and anaerobically digested sludge (eggs added before or after digestion) and a 3% aqueous suspension of top soil. The viability of the Ascaris eggs they did recover decreased rapidly in all samples stored at 25°C, and the authors stated that 10 to 16 months of storage at 25°C was sufficient to render most of the recovered eggs nonviable. In samples stored at 4°C or in the ground, viable eggs were recovered in some samples after 24 to 33 months of storage. While the study of O'Donnell, et al.(1) is quite different than the present lagoon study, it is of interest to note that their findings on the inactivation of Ascaris eggs at 25°C are similar to the results observed in the sludges stored in two large columns in the present study where complete inactivation was observed between 12 and 15 months of storage. In the study of O'Donnell, et al.(1), the recovery of eggs from samples was very low at 3 months of storage and thereafter, while in the present lagoon study, the percent recovery of eggs from samples did not decrease dramatically after storage but decreased gradually over time. Since different recovery procedures were used in the two studies, it is not possible to explain this difference.

As mentioned above, the more rapid die-off of Ascaris eggs in the Port Aransas lagoon as compared to the New Orleans lagoon was likely due to the petroleum residues in the Texas sludge. The organic content of the petroleum levels was in the order of 20% with high contents of:

Naphthalene
Phenanthrene
Bi ≤ (2-ethylhexyl)phthalate
2-Methylnaphthalene
1-Methylnaphthalene
2,6,10,14-Tetramethylpentadiene
Pristine
Isoprenoid
Phytene
Phthalene

In a review by Japanese researchers, complete Ascaris egg inactivation was reported to occur following exposure to many oils and petroleum related compounds (Table 31).

Morishita (47) indicated in his review that organics having the following characteristics were more effective in inactivating parasites:

1. low polarity
2. high volatility
3. low water solubility

It should be noted that these are the general properties of petroleum residues. In his review, Morishita reported the effectiveness of certain compounds in inactivating Ascaris eggs as shown in Table 32.

This indicates the possible role of organics in the high die-off of Ascaris eggs in the Texas sludge. Refinery and petroleum contaminated sludges that are not hazardous could possibly be mixed with municipal sludges to enhance the sludge disinfection process for parasite inactivation in lagoon stored sludge. A non-hazardous sludge is a sludge that passes the Toxicity Characteristics Leaching Procedure (TCLP).

SLUDGE SETTLING EXPERIMENT

The settling test on a thickened anaerobically digested sludge (about 4% solids) spiked with Ascaris eggs was conducted to determine the relationship of the settling of Ascaris eggs and the settling of solids. As expected, the eggs eventually settled to the bottom of the sludge column, but some eggs could be found still in the supernatant in the top one-half of the sludge column (top 0.91m (3 ft) of a 1.82m (6 ft) column) after one week (Tables H-15, Appendix H). Cram (26) found Ascaris eggs in the upper one-third of a 0.66m (26 in) long column of primary sewage after only 15 minutes of settling. It would appear that Ascaris eggs settle more slowly in an anaerobic sludge than in raw sewage. This is to be expected since the anaerobic sludge is thicker.

Although Ascaris eggs are expected to be associated with the sludge rather than the effluent in a sewage treatment plant, eggs have been

TABLE 31. COMPOUNDS THAT EFFECTIVELY INACTIVATE *ASCARIS* EGGS[a] (MORISHITA, 1972) (47)

Compound	Concentration	Exposure Time
beta-naphtol	0.1%	---
alpha-toluidine	1.0%	---
pyridine	0.1%	---
furfrol	1.0%	---
ethyl Iodine	0.1%	---
aldline	1.0%	---
dieldrine	0.1%	---
pentachlorophenol	1.0%	---
acetoacetic acid	0.1%	---
undecylenic acid	1.0%	---
phenolanline	0.15%	---
dimethylaniline	0.1%	---
benzyl chloride	0.1%	---
D-D (propyldichloride and propylene dichloride)	0.1%	1 day
p-thiocresol	0.05%	6 days (9 days at 20°C and 7 days at 30°C)
p-chloro-m-cresol	0.5%	10 days
theionenol	0.05%	8 days
lethane (an insecticide made in USA)	0.05%	8 days
sulfonated oil (Turkey red oil)	1.0%	---

[a] Embryonation of eggs completely inhibited
[b] Time not mentioned

recovered from the effluent. Reimers *et al*. (7) reported finding eggs in the effluent of a plant in Alabama, and Fox *et al*. (10) found eggs in the effluent from a wastewater treatment plant in Chicago. Whether eggs occur in the effluent from a treatment plant is dependent on the settling time and the solids content of the sludge.

STATISTICAL ANALYSIS

The abiotic parameters and the inactivation of the different microorganisms were analyzed to determine whether any of the parameters could be used as an indicator of pathogen inactivation. None of the

TABLE 32: EFFECTIVENESS OF ORGANIC COMPOUNDS ON *ASCARIS* EGG INACTIVATION (47)

Organics	Effectiveness In Inactivating *Ascaris* eggs
Chloroform, ether, benzol and carbon disulfide	good (Total inactivation in 1 month)
Toluol, carbon tetrachloride, xylol, phenols, and ethyl acetate	moderate (Total inactivation in 1 year)
A-butanol, alcohol, creosote and acetone	low effectiveness (Little inactivation in 1 year)

abiotic measurements of the sludge sediment could be related to the inactivation of any of the indicator bacteria or pathogens. However, in the case of the sludge supernatant, the inactivation of the bacterial indicators was related statistically to time, COD, total solids, and volatile solids. These data indicate an influence of oil and grease on the settling of bacterial solids.

In the case of Port Aransas lagoon, the abiotic parameters, time, pH, total solids and volatile solids were significantly related to virus inactivation. This indicated that virus inactivation was probably related to the organics contaminants. Virus inactivation in the New Orleans lagoon was not significantly related to any abiotic parameters.

The reduction of *Ascaris* viability in the Port Aransas lagoon was not correlated with any abiotic parameters, and the faster die-off was apparently due to inactivation by the petroleum organics. The observations on New Orleans lagoon sludge indicate that inactivation was correlated only with volatile solids. No significance was noted with respect to the supernatant abiotic factors and inactivation of *Ascaris* eggs.

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APPENDIX A

VIRUS ANALYSIS PROTOCOL

Method for Elution and Concentration of Viruses from Sludge Solids

Berman and Safferman 1981 (42)

1. To 100 mL of sludge add 1.0 mL of 0.05m $AlCl_3$, then lower the pH of the mixture to 3.5 using 5M HCl. Mix this combination on a magnetic stirrer for 30 minutes.
2. Centrifuge mixture for 15 minutes at 2500 xg.
3. Resuspend the solids in 100 mL of buffered 10% Beef extract and then stir the mixture on a magnetic stirrer for 30 min.
4. Centrifuge the mixture for 30 min. at 8300 xg and collect the supernatant.
5. Filter the supernatant through P (Filterite Duo-Fine Series) 3.0, 0.45, 0.25um Millipore Filter.
6. Dilute the filtrate by adding 230 mL of sterile distilled water per 100 mL of filtrate or 2.3 mL H_2O per 1.0 mL of filtered eluate.
7. Lower the pH of the diluted filtrate to 3.5 by addition of 1M HCL, then stir the mixture on a magnetic stirrer for 30 min.
8. Centrifuge the mixture for 10 min. @ 2500 xg and collect the pellet.
9. Resuspend the pellet in 5.0 mL of 0.15 M Na_2 HPO_4 buffer at 7.2.

Procedure for Titrating Viruses

Inoculating Virus-containing Sample onto Cell Cultures

1. From one to four hours before cultures are to be inoculated, replace medium in culture vessels with an equal volume of maintenance medium.

2. Maintain cultures at 36.5° ± 1° C until they are to be inoculated (page 108).

3. Identify individual culture vessels on their outsides with an indelible marker before they are inoculated.

4. Pour medium from cell culture vessels, and discard medium.

5. Carefully inoculate into each culture vessel the volume of sample which is correct for vessels of that size.

   See Table A-1 for inoculum sizes appropriate for commonly used cell culture vessels.

6. Immediately rock innoculated culture vessel gently to achieve uniform distribution of sample on surface of cell monolayer.

   Uniform distribution of sample inoculum results in uniform distribution of plaques and thereby facilitates accurate plaque counts.

7. Incubate inoculated cell cultures for two hours at room temperature (22-25° C) to permit viruses to absorb onto and infect cells.

8. Apply warm (-46° C) agar overlay medium (page 96) to each culture vessel.

   See Table A-1 for the amount of overlay medium that should be added. Pour medium onto side of cell culture vessel opposite the cell monolayer, allow medium to cool momentarily, and then place the culture vessel, monolayer side down, on a stationary table or bench at room temperature (22-25° C) so that the agar will distribute evenly as it solidifies.

9. Cover cell culture vessels with a sheet of aluminum foil, a tightly woven cloth, or some other suitable cover to prevent photoinactivation of virions.

   Agar begins to harden almost immediately and fully solidifies in 30 to 60 minutes.

10. Invert culture vessels and incubate in the dark at 36.5° ± 1° C.

Counting Viral Plaques

1. Begin counting and marking plaques in cultures two days after overlaying, and repeat procedure every two days for a total of 10 days (for enteroviruses) after overlaying.

2. Record plaque counts at each reading.

3. Calculate virus titers (plaque-forming units (PFU) for each inoculated virus-containing sample).

   Total number of plaques per culture vessel (or average number of plaques per culture vessel if several vessels have been inoculated with the same sample) to obtain the virus titer of sample in terms of PFU per inoculum volume. To obtain PFU per mL, multiply the number of PFU by the reciprocal of the inoculum volume.

TABLE A-1. GUIDE FOR DETERMINING VOLUME OF CELL CULTURE MEDIUM, VIRUS SAMPLE INOCULUM, AND OVERLAY MEDIUM TO BE USED WITH VARIOUS SIZED CELL CULTURE VESSELS

Vessel Type and Size (Flat Sided Glass or Plastic Bottle)	Volume of Fluid in mL		
	Growth or Maintenance Medium	Virus Sample Inoculum	Agar Overlay Medium
1 oz.[a]	4	0.1	5
2 oz. or 25 cm^2	8	0.2	10
4 oz.	12	0.3-0.4	15
6 oz.	15	0.5-1.0	20
8 oz. or 75 cm^2	20	0.5-1.5	25
16 oz. or 150 cm^2	40	1.0-3.0	50
32 oz.	50	not commonly used	not commonly used
Roller Apparatus (Glass or Plastic Bottle)			
64 oz.	100	cannot be used	cannot be used
Cell Culture Tube (Glass or Plastic)			
16 x 150 mm	2	cannot be used	cannot be used

[a] Vessels designated in oz. are commercially available only in oz.

Procedure for Preparation of BGM Cell Cultures

A. General Procedures

The BGM cell line grows readily on the inside surfaces of plastic flat-sided vessels and glass bottles and test tubes. BGM cell cultures can be purchased from several companies for plaque assay or plaque confirmation procedures. Although it is labor intensive for a laboratory to maintain the BGM cell line and to prepare cultures, it is much less expensive to prepare cultures than to purchase them.

1. Pass and maintain BGM stock cultures in 16 to 32 oz.* (or equivalent in growth area), flat-sided, glass bottles or in plastic cell culture flasks.

   If available, roller bottles and roller apparatus units are preferable to flat-sided bottles or flasks for growing cells because roller cultures require less medium than flat-sided bottles per unit of cell monolayer surface. For growing cells in roller bottles, adjust roller apparatus rotation speed to one-half revolution per minute.

2. Prepare cell cultures for plaque assays in vessels with growth areas of 45 sq. cm (6 oz.) or larger.

   Vessels with relatively large surface areas are used to accomodate the large sample inoculums in environmental virology studies. Vessels with smaller surface areas may be used, if necessary. Only flat-sided cell culture vessels can be used for plaque assays.

3. Prepare cultures for plaque confirmation in 16 x 150 mm glass or plastic cell culture tubes.

4. Except during handling operations, maintain BGM cells at 36.5° ± 1° C in cell culture vessels that are air-tight.

   (a) Maintain roller bottles that contain cells in constant motion.

   (b) Maintain flat-sided cell culture bottles or flasks that contain cells in a stationary position with the flat side (cell monolayer side) down.

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* Vessel size is designated in oz. when commercially available only in such designations.

(c) Maintain cell culture tubes in either stationary or rotating racks slanted upwards at an angle of approximately 15° so that the fluid inside extends upward 1/3 to 2/3 the length of the tube.

Cell culture vessels must be stored in a way such that their liquid contents do not touch the inner surface of the vessels' caps.

5. To reduce shock to cells, warm growth medium, maintenance medium, washing solution used for removing toxic materials from inoculated cell cultures, and all other solutions to 36.5° ± 1° C before placing them on cell monolayers.

6. Test all media and solutions to be used in cell culture operations to assure their microbiological sterility.

7. Introduce only trypsin-EDTA solution and sample inoculums directly onto the monolayer surface.

   Inoculums are introduced onto the monolayer surface directly to minimize loss of viruses through adherance.

8. Introduce all materials other than trypsin-EDTA solution or inoculum onto a part of the inside container wall that is not covered by cell monolayer surface.

   This precaution is taken to dissipate force used to introduce fluids.

9. Pass stock BGM cell cultures at approximately seven-day intervals.

10. Prepare cultures for plaque assay and plaque confirmation three to seven days before cultures are to be inoculated with virus-containing material.

B. Procedure for Passage of BGM Cells

1. Pour spent medium from cell culture vessels, and discard the medium.

2. Add to the cell cultures a volume of warm (36.5° ± 1° C) trypsin-EDTA solution equal to 40% of the volume of medium replaced (See Table 8-1).

   Pour the trypsin-EDTA solution (xtrypsin-EDTA, Flow Laboratory, McLean, VA) directly onto the cells.

3. Allow trypsin-EDTA solution to remain in contract with the cells until cell monolayer can be shaken loose from inner surface of

cell culture vessel (about 10 minutes).

If necessary, a sterile rubber policeman (or scraper) may be used to physically remove the cell sheet from the bottle. However, this procedure should be used only as a last resort because of the risk of cell culture contamination inherent in such manipulations.

4. Pour the suspended cells into centrifuge tubes or bottles.

   To facilitate collection and resuspension of cell pellets, use tubes or bottles with conical bottoms. Centrifuge tubes and bottles used for this purpose must be able to withstand the g-force applied.

5. Centrifuge cell suspension at 1,000 x g for 10 minutes to pellet cells.

6. Pour off and discard the supernatant.

7. Suspend the pelleted cells in growth medium.

   The quantity of medium used for resuspending pelleted cells varies from 10 mL to more than 1 liter, depending upon the volume of the individual laboratory's need for cell cultures. Resuspend passaged cells in large volumes of medium to allow thorough mixing of cell pellets (to reduce sampling error) and to minimize the significance of the loss of the 1 mL of cell suspension required for the cell counting procedure. Do not dilute cells to a concentration of less than 6 x 10^5 per mL, because viable cell counts cannot be done accurately with lesser concentrations of cells.

8. Perform a viable cell count on this concentrated suspension.

9. Dilute the cell suspension in growth medium to a concentration of approximately 2.5 x 10^5 viable cells per mL of medium.

10. Dispense the cell suspension into cell culture vessels.

    The quantities of suspension that must be used for culture vessels of different sizes are shown in Table A-1.

C. Maintenance Medium

Formula (Preparation of 1 liter)

If medium is to be used for washing cells before cell cultures are to be inoculated with viruses, replace 50 mL of fetal calf serum with 50 mL of distilled water.

Distilled water	850.0 mL
Earle's BSS 10X stock	100.0 mL
Phenol red, 0.5%	1.0 mL
$NaHCO_3$, 7.5%	7.0 mL
Fetal calf serum	.50.0 mL
Penicillin-streptomycin (5000 in u/mL/-5000 mg/mL)	1.0 mL

Procedure

(a) Filter through a 0.22 um membrane any ingredient in the formula that is not sterile.

(b) Place 850 mL of distilled water into a clear glass vessel.

(c) Maintain continuous stirring.

(d) Add Earle's BSS 10X stock, phenol red, and 7.5% $NaHCO_3$ to the distilled water.

(e) Store medium at $4^{o}C$ until needed.

(f) Add fetal calf serum and antibiotics to medium immediately before medium is used.

2X Eagle's Minimal Essential Medium Without Phenol Red (for use in Overlay Medium)

Formula (Preparation of 1 liter)

Solution A

Distilled water	625 mL
Hanks' BSS 10X stock	200 mL
Vitamins 100X stock	20 mL
Amino acids 100X stock	20 mL

Solution B

Distilled water	50 mL
L-Tyrosine	146 mg

Solution C

Distilled water	50 mL
L-Cysteine	96 mg
NaOH, 1N	As needed

Procedure

(a) Place 625 mL of distilled water into a clear glass vessel.

(b) Maintain constant stirring.

(c) Add to the distilled water the ingredients for Solution A.

Allow each ingredient to go into solution before adding the next ingredient.

(d) Dissolve 146 mg of L-tyrosine into 50 mL of distilled water (Solution B).

Boil over bunsen burner or hot plate until the L-tyrosine goes into solution.

(e) Cool Solution B, and add Solution B to Solution A.

(f) Dissolve 96 mg of L-cysteine into 50 mL of distilled water, and add 1N NaOH until L-cysteine is neutralized (Solution C).

(g) Add Solution C to Solutions A and B.

(h) Adjust volume of medium (Solutions A, B, C) to 1 liter with distilled water.

(i) Filter-sterilize medium through a 0.22 um membrane.

(j) Store medium at 4°C.

Agar Overlay Medium

Formula (Preparation of 1 liter)

Mixture A

2X Eagle's MEM without phenol red for use in overlay medium	415.0 mL
Fetal calf serum	20.0 mL
$NaHCO_3$, 7.5%	30.0 mL
$MgCl_2$, 1%	10.0 mL
Neutral red, 0.1%	15.0 mL
Penicillin-streptomycin, stock solution	1.0 mL
Tetracycline, stock solution	0.5 mL
Fungizone, stock solution	0.2 mL

Mixture B

Agar	15.0 gm
Distilled water	500.0 mL

Procedure

Do not prepare Mixtures A and B in advance of the day on which they are to be used.

(a) Mixture A

1. Filter through a 0.22 um membrane any ingredient in the formula that is not sterile.

2. Place 415 mL of 2X Eagle's MEM without phenol red into a clear glass vessel.

3. Maintain constant stirring.

4. Add the fetal calf serum, $NaHCO_3$, $MgCl_2$, neutral red, and antibiotics to the 2X Eagle's MEM (Mixture A).

5. Warm mixture A to $36.5^{\circ} \pm 1^{\circ}C$ in a water bath.

If Mixture A is prepared more than one hour before it is to be used, store it at 4°C, and warm it to 36.5° ± 1°C about 30 minutes before it is needed.

(b) Mixture B

1. Place 500 mL of distilled water into a glass vessel that can withstand autoclaving.

2. Add the agar to the distilled water.

3. Autoclave agar and water at 121°C for 15 minutes.

4. Cool dissolved agar to 56° ±1° C in a water bath.

(c) Immediately before overlay medium is to be placed on cell cultures, combine Mixtures A and B and add 10 mL of sterile whole milk.

(d) Mix quickly by swirling.

If a large number of cell cultures is to be overlayed, maintain medium in a 36.5° ± 1° C water bath during overlay procedure.

(e) Overlay cells immediately.

See Table A-1 for quantity of overlay required.

Eagle's Minimal Essential Medium (MEM) with Hanks' Balanced Salt Solution

Formula (Preparation of 1 liter)

This medium may also be prepared as a ten-fold (10X) concentrate, and components of the formula may be prepared in even higher concentrations (See below). Formulations for preparing this medium in 1X and 2X concentrations (the latter for use in preparing overlay medium for plaque assays) from more concentrated sub-components are presented in later sections.

Distilled water	750.0 mL
Inorganic salts	
$CaCl_2$	140.0 mg
KCl	400.0 mg
KH_2PO_4	60.0 mg
$MgCl_2.6H_2O$	100.0 mg

$MgSO_4.7H_2O$	100.0 mg
NaCl	8000.0 mg
Na_2HPO_4	60.0 mg

Amino Acids

L-Arginine HCl	84.0 mg
L-Cystine	48.0 mg
L-Glutamine	300.0 mg
Glycine	30.0 mg
L-Histidine HCl-H_2O	42.0 mg
L-Isoleucine	105.0 mg
L-Leucine	105.0 mg
L-Lysine HCl	146.2 mg
L-Methionine	30.0 mg
L-Phenylalanine	66.0 mg
L-Serine	42.0 mg
L-Threonine	95.0 mg
L-Trypotophan	16.0 mg
L-Tyrosine	73.0 mg
L-Valine	93.6 mg

Vitamins

Choline chloride	4.0 mg
Folic acid	4.0 mg
i-Inositol	7.0 mg

Nicotinamide	4.0 mg
Panthothenic acid	4.0 mg
Pyridoxal HCl	4.0 mg
Riboflavin	0.4 mg
Thiamine HCl	4.0 mg

Other components

Glucose	1000.0 mg
Phenol red	5.0 mg

Procedure

(a) Place 750 mL of distilled water into a clear glass vessel.

(b) Maintain constant stirring.

(c) Add the ingredients listed to the distilled water.

Allow each ingredient to go into solution before adding the next ingredient.

(d) Adjust the volume of the solution to 1 liter with distilled water.

(e) Filter -sterilize medium through a 0.22 um membrane.

(f) Store medium at 4°C.

APPENDIX B

PROCEDURES FOR PARASITOLOGIC EXAMINATION OF STORED-SLUDGE SAMPLES

PROCEDURE A:

REAGENTS, MATERIALS AND EQUIPMENT

Reagents

1. 1% solution of "7X" (v/v), an anionic detergent
2. $MgSO_4$ Soln.; specific gravity 1.20, adjusted with hydrometer
3. Alcohol-Acid Soln.; 0.27% H_2SO_4 (v/v) in 35% Ethyl-Alcohol
4. Ether, anhydrous
5. Culture fluid; 0.1 N H_2SO_4 (0.27% H_2SO_4, V/V, in water)
6. 0.25% Sodium Citrate Soln. (2.5 gm sodium citrate/L saline)

Materials

1. Beakers, 1000 mL., tall form, Berselius, Pyrex
2. Beakers, 1000 mL., low form, Kimax
3. Sieves, 7 in., gauges 10, 20, 50, 100, and 150
4. Funnel, powder filling, 150 mm, Nalgene
5. Hydrometer, sp. gr. 1.0 - 1.3
6. Centrifuge tubes, conical, polypropylene, 50 mL
7. Centrifuge tubes, conical, Pyrex, 15 mL
8. Pipettes, Pasteur, disposable, 5 3/4 in.
9. Microscope slides, glass, 3 x 2 in.
10. Cover glasses, 22 x 30, 24 x 40, 24 x50 mm
11. Wash bottles, plastic, 500 mL and 1000 mL

12. Culture dishes or vials.

Equipment

1. Centrifuge, IEC, with swinging bucket head and cups for 50 mL tubes
2. Centrifuge, Clinical model, with swinging bucket head for 15 mL conical tubes
3. Magnetic stirrer
4. Ultrasonic cleaner, 11 1/2" x 5 1/2" x 6"
5. Vortex mixer
6. Microscope, compound, binocular

ANALYSIS PROCEDURE

1. Place 100 mL of a thoroughly mixed sludge sample into a 1000 mL beaker, add 1% "7X" to about 400 mL, and stir for 5 minutes on a magnetic stirrer.
2. Strain homogenized sample through a 20-mesh sieve into a beaker. This sieve is placed on a funnel over 1000 mL beaker and the sample washed through the sieve with aid of stream from a squirt bottle containing 1% "7X".
3. After sample has been passed through the sieve, it is allowed to settle for at least one hour.
4. Decant and discard supernatant; mix sediment and distribute equally to four 50 mL centrifuge tubes, bring volume in each tube to 50 mL with water.
5. Centrifuge for 5 minutes at 800g and pour off supernatant from each tube. (The packed sediment in each tube should not exceed 5 mL; if so, add water, distribute evenly among additional tubes, repeat centrifugation, and decant.)
6. Add 5 to 10 mL of $MgSO_4$ solution to packed sediment of each tube and mix for 15 to 30 seconds on a Vortex mixer (use care not to cause splashing of mixture from tube.)
7. Add additional $MgSO_4$ solution to each tube to bring to 50 mL and centrifuge for 5 minutes at 800g.

8. After the centrifuge has come to a complete stop, pour the top 25 to 35 mL of supernatant from each tube through a 150 mesh sieve into a 400 mL beaker. Rinse the sieve with water from a squirt bottle and add water until volume is about 300 mL and mix by stirring.

9. Distribute the diluted supernatant into eight 50 mL centrifuge tubes taking care to thoroughly rinse beaker into tubes, centrifuge for 5 minutes at 800g and discard the supernatant.

10. Transfer the sediment from the tubes to one or more 15 mL conical centrifuge tubes, using stream of water from a squirt bottle to completely rinse all sediment from each tube. Fill to near top with water.

11. Centrifuge from 3 minutes at 800g. Decant and discard supernatant. (If sediment exceeds 1.5 mL, dilute in water, distribute to one or more additional tubes as required and centrifuge again.)

12. Add 2 mL of alcohol-acid solution to packed sediment and mix thoroughly on a Vortex mixer, add 8 mL of alcohol-acid solution.

13. Add 3 mL of ether, cap, and shake vigorously for 30 seconds.

14. Centrifuge at 800g for 3 minutes.

15. Using an applicator stick, loosen the plug at the interface between the ether layer and the alcohol-acid layer by ringing the inner circumference of the tube and pour off the entire supernatant including plug by quickly inverting the tube. Touch tip of tube to paper towel to remove excess fluid and to prevent it from draining back into the tube.

16. Add 2-3 mL of culture fluid to sediment in tube(s), mix on Vortex mixer then bring volume to 15 mL by adding more of culture fluid.

17. Centrifuge for 3 minutes at 800g and pour off supernatant.

18. Add a few drops of culture fluid to sediment, mix with clean pipette, and transfer to culture dish. Rinse tube and pipette with a 1 mL or less of culture fluid. Add additional culture fluid to culture dish; if necessary, to bring depth of fluid to 3 to 4 mm.

19. Cover culture dishes and store in dark or subdued light at 22-28°C. Dishes should be agitated every few days to mix sediment and aerate fluid.

20. After a minimum of 28 days, the cultured sample is transferred to

one or more 15 mL conical centrifuge tubes depending on volume of sample.

21. Centrifuge at 800g for 3 minutes and then pour off supernatant.

22. With pipette, transfer sediment to one or more slides for microscopic examination. Transfer appropriate amount of sediment for size of coverglass (20 x 30 mm or 22 x 40 mm recommended). Fluid should not extend beyond edge of coverglass.

23. Systematically examine each egg present and record whether it contains a developed larva (viable) or is undeveloped (non-viable); also record numbers of all other helminth eggs present and their state of development.

PROCEDURE B:

REAGENTS, MATERIALS, AND EQUIPMENT

Reagents

1. 1% solution of "7X" (v/v), an anionic detergent

2. $MgSO_4$ Soln.; specific gravity 1.20, adjusted with hydrometer

3. Sodium Hypochlorite, 0.6% solution of sodium hypochlorite (v/v) or 10.0% Clorox^R (v/v)

4. Culture fluid; 0.5% formalin (5 mL formalin and 995 mL H_2O)

5. Prosil-28, an organo silane surface-treating agent

6. 0.25% sodium citrate Soln.

Materials

1. Beakers, 1000 mL., tall form, Berselius, Pyrex

2. Sieve, 20 mesh, 5 inch diameter

3. Sieve, 150 mesh, 5 inch diameter

4. Sieve, 400 mesh screen attached to bottom of cut off stainless steel funnel

5. Funnel, powder filling, 150 mm, Nalgene

6. Funnel, Pyrex, 50 mm with 1/2 inch stem

7. Hydrometer, sp. gr. 1.0 - 1.3
8. Centrifuge tubes, conical, polypropylene, 50 mL
9. Centrifuge tubes, conical, Pyrex, 15 mL
10. Pipettes, Pasteur, disposable, 5 3/4 in.
11. Microscope slides, glass, 3 x 2 in.
12. Wash bottles, plastic, 50 mL and 1000 mL
13. Spray bottle, all purpose household sprayer
14. Culture dishes or vials
15. Parafilm

Equipment

1. Centrifuge, IEC, with swinging bucket head and cups for 50 mL tubes
2. Centrifuge, Clinical model, with swinging bucket head for 15 mL conical tubes
3. Magnetic stirrer
4. Ultrasonic cleaner, 11 1/2" x 5 1/2" x 6"
5. Vortex mixer
6. Microscope, compound, binocular
7. Waring blender with 40 fl. oz. container
8. Reostat (Powerstat)

ANALYSIS PROCEDURE

1. Blend a measured weight or volume of sludge (30-50 g wet weight or 50-100 mL volume) with 200 mL water for one minute at high speed. Pour the blended sample into a 1000 mL tall form beaker and add 1% "7X" to 900 mL.
2. Let settle for 3 hours, pour off the supernatant, add water to 300 mL and blend again for 1 minute at high speed. (While

settling, the solution may be occasionally stirred to ensure that any material floating on the surface will settle).

3. After blending for the second time, allow to settle for 1 hour in 1% "7X".

4. Pour off the supernatant and discard. Add 300 mL of 1% "7X" and stir for 5 minutes on a magnetic stirrer.

5. Strain homogenized sample through a 20-mesh sieve into a beaker. The sieve is placed on a funnel over 1000 mL beaker and the sample washed through the sieve with aid of stream from a squirt bottle containing 1% "7X" or spray bottle containing H_2O.

6. After sample has been passed through the sieve, it is allowed to settle for at least one hour.

7. Decant and discard supernatant; mix sediment and distribute equally to four 50 mL centrifuge tubes, bring volume in each tube to 50 mL with water.

8. Centrifuge for 5 minutes at 800g and pour off supernatant from each tube. (The packed sediment in each tube should not exceed 5 mL: if so, add water, distribute evenly among additional tubes, repeat centrifugation, and decant.)

9. Add 5 to 10 mL of $MgSO_4$ to packed sediment of each tube and mix for 15 to 20 seconds on a Vortex mixer (use care not to cause splashing of mixture from tube).

10. Add additional $MgSO_4$ solution to each tube to bring to 50 mL and centrifuge for 5 minutes at 800g.

11. A 150 mesh sieve and funnel are placed on a ring stand with the 400 mesh sieve placed directly under the funnel to collect the sample.

12. After the centrifuge has come to a complete stop, pour the top 25 to 35 mL of supernatant from each tube through the 150 mesh sieve. Using a spray bottle containing H_2O or a squirt bottle containing 1% "7X", wash the sample through the 150 mesh sieve and funnel.

13. Sample collected on the 400 mesh sieve is washed using a combination of directed jet spray and coarse spray from spray bottle onto upper surface of the sieve. (Use care not to cause splashing of sample from sieve.)

14. If sediment collected on 400 mesh sieve is less than 1 mL, rinse the sediment into a 15 mL conical centrifuge tube by directing a

stream of water from a squirt bottle (or coarse spray from spray bottle) onto the bottom surface of the sieve that has been inverted above a small glass funnel placed on top of the 15 mL conical centrifuge tube. (If sediment collected on 400 mesh sieve is greater than 1 mL, substitute the 15 mL centrifuge tube with a 50 mL centrifuge tube(s). Then, centrifuge the tube(s) for 5 minutes at 800g, discard supernatant and transfer sediment to one or more 15 mL conical centrifuge tubes.)

15. If necessary, fill 15 mL conical centrifuge tube to near top with water. Centrifuge for 3 minutes at 800g, discard supernatant.

16. If sample is not to be treated with a fungicidal agent before culturing, go to step no. 21.

17. Add 2-3 mL of 0.6% sodium hypochlorite to sediment in tube(s), mix on Vortex mixer, then bring volume to 10 mL by adding more hypochlorite solution. Let sample stand for 10 minutes.

18. Fill tube(s) to near top with water. Centrifuge for 3 minutes at 800g, discard supernatant.

19. Wash sediment with water to remove hypochlorite by mixing sample with H_2O, centrifuging, and discarding supernatant.

20. Repeat step no. 19.

21. Add 2-3 mL of culture fluid (0.5% formalin) to sediment in tube(s), mix on Vortex mixer, then bring volume to 15 mL by adding more culture fluid.

22. Centrifuge for 3 minutes at 800g and pour off supernatant.

23. If sample is not to be microscopically examined before culturing, go to step no. 29.

24. With clean pipette, transfer sediment to one or more slides for microscopic examination. Transfer appropriate amount of sediment for size of coverglass used (20 x 30 mm or 22 x 40 mm recommended). Fluid should not extend beyond area of coverglass.

25. Systematically examine each preparation under at least 100x magnification of a compound, binocular microscope.

26. Count all Ascaris eggs present and record whether they are viable (contains motile larva, or ovum or developing larva that appear to be alive) or non-viable (contains obviously dead larva or ovum); also, record numbers of all other helminth eggs present and their state of development.

27. After the eggs have been counted, they are washed off the microscope slide (or slides) with a stream of culture fluid from a squirt bottle into a 15 mL conical centrifuge tube. If needed fill tube to near top with culture fluid.
28. Centrifuge for 3 minutes at 800g and pour off supernatant.

29. Add a few drops of culture fluid to sediment, mix with clean pipette, and transfer to culture dish. Rinse tube and pipette with 1 mL or less of culture fluid. Add additional culture fluid to culture dish, if necessary, to bring depth of fluid to 3 to 4 mm.

30. Cover culture dishes and store in dark or subdued light at 22-28°C. Dishes should be agitated every few days to mix sediment and aerate fluid. Culture dishes may be covered with parafilm to prevent splashing of fluid out of dish.

31. After a minimum of 28 days, the cultured sample is transferred to one or more 15 mL conical centrifuge tubes depending on volume of sample.

32. Centrifuge for 3 minutes at 800g and discard supernatant.

33. Examine by steps no. 24-26.

PROCEDURE FOR TESTING INFECTIVITY OF ASCARIS EGGS RECOVERED FROM SLUDGE

The Ascaris eggs recovered from one or more sludge samples collected from a lagoon, column or distilled water control at each sampling time are tested for infectivity on mice after they have been cultured for 28 or more days.

PROCEDURE

1. After the cultured eggs have been counted (see #26 in procedure B), they are washed off the microscope slide (or slides) with a stream of water from a wash bottle into a 15 mL centrifuge tube. The eggs will be determined to contain an infective stage larva by noting the presence of a molted cuticle around the larva within the eggs.

2. When all of the eggs from one sample have been collected in the tube, they are suspended in water and centrifuged for 2 minutes at 800g.

3. The supernatant is discarded, the sediment resuspended in water, and the tube centrifuged.

4. The supernatant is discarded, and this sediment is used as inoculum for one or more mice.

5. The inoculum for each mouse should contain about 300 infective eggs. If more than 300 infective eggs are present in the sediment recovered from the soil sample, it is divided into two or more inocula so that each will contain approximately 300 infective eggs.

6. If less than 300 eggs are present in the sediment from one sample, then the infective eggs from two or more samples are combined to produce an inoculum of about 300 eggs. In any case, a minimum of four mice will be inoculated from samples collected at each sampling time.

7. If eggs are still corticated after each inoculum has been prepared, it is suspended in a solution of 1% sodium hypochlorite for about 15 minutes to remove the outer shell of the egg to facilitate the hatching of the larvae after they are inoculated into a mouse.

8. The suspension is centrifuged at 800g, the supernatant discarded and then the sediment is washed once in water to remove all of the hypochlorite.

9. The sediment containing the appropriate number of eggs is suspended in about 0.3 mL of normal saline and then is fed by stomach tube to a mouse (Strain CD 1, approx. 8 wks old).

10. At 48 hours after inoculation, each mouse is killed by cervical dislocation and the liver removed, rinsed in normal saline and processed by the method of Johnstone, et al.(48), 1978, as follows:

11. The entire liver is homogenized in a Waring Blender at high speed for 20 seconds in 40 mL of 0.25% sodium citrate in saline with two drops of octyalcohol.

12. Suspension is strained through three layers of gauze into a beaker.

13. The strained suspension is transferred to a 50 mL centrifuge tube and centrifuged at 1,000g for 60 seconds. The supernatant is then discarded.

14. The sediment is resuspended in the citrate-saline solution and centrifuged at 500g for 20 seconds; supernatant is discarded.

15. Step no. 14 is repeated.

16. The sediment is transferred to glass slides and examined under 40X magnification.

CONTROLS

1. Eggs recovered from the feces of pigs are placed into cultures directly and these are used to infect mice at same time as mice are infected with eggs from test samples. Four or more control mice will be infected at each sampling.

2. Eggs from culture are processed by the procedure listed above for eggs recovered from the lagooned sludge.

3. Control cultures will be at least 28 days old and no more than 9 months old.

PROCEDURES FOR THE PARASITOLOGIC EXAMINATION OF DISTILLED WATER CONTROLS

PROCEDURE

1. The contents of the vial containing the eggs in distilled water are transferred to a 50 mL centrifuge tube and the vial thoroughly rinsed into the tube with the aid of a stream of water from a squirt bottle. If needed, the 50 mL centrifuge tube is filled to near top with water.

2. Centrifuge for 5 minutes at 800g and pour off supernatant from the tube.

3. Rinse the sediment from the 50 mL centrifuge tube into a 15 mL conical centrifuge tube with the aid of a stream of water from a squirt bottle. If needed, fill the 15 mL conical centrifuge tube to near top with water.

4. Centrifuge for 3 minutes at 800g, then discard the supernatant.

5. Continue at step #16 of Procedure B for the Parasitologic Examination of Lagoon-Stored Sludge.

APPENDIX C

ABIOTIC SLUDGE LAGOON DATA

TABLE C-1. ABIOTIC DATA ON THE ANAEROBIC SLUDGES BEFORE DISPOSAL TO NEW ORLEANS LAGOON FOR 2/6/84

Sample No.	Temp (^oC)	Conductivity (um Hos)	pH (S.U.)	ORP (MV)	Total Solids (g/L)	Volatile Solids (g/L)	% Volatile of total Solid	COD (mg/L)	NH_3 (mg/L)
11	11.0	2700	6.9	-214	40.92	18.62	45.5	42,000	50
12	12.5	2800	6.8	-246	47.32	21.49	45.4	42,500	44
21	13.0	2950	6.8	-238	38.42	17.70	46.1	34,000	50
22	13.0	2800	6.8	-251	53.88	25.25	46.9	49,000	47
31	12.5	2850	6.9	-230	38.36	17.67	46.1	34,000	44
32	12.5	2800	6.8	-234	40.71	19.23	47.2	35,500	44
41	12.5	2850	6.8	-260	41.89	19.40	46.3	39,500	44
42	13.0	2800	6.8	-265	62.33	29.77	47.8	54,000	58
51	12.0	2950	6.8	-265	34.30	15.65	45.6	34,000	42
52	12.5	2850	6.8	-260	56.17	26.27	46.8	51,000	50

TABLE C-2. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 3/12/84

Sample Number	pH (s.u.)	Temp. (^oC)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	7.2	15.0	-60	2600	0.1433	0.0328	650	244
21	7.2	14.0	-80	2600	0.1411	0.0374	650	250
31	7.2	14.0	-60	2600	0.1396	0.0383	600	246
41	7.2	14.0	-70	2600	0.000	0.0349	630	246
51	7.3	14.0	-90	2600	0.1329	0.371	600	298
12	6.8	14.0	-240	2700	4.253	1.967	35,000	420
22	6.8	14.0	-220	2700	5.846	2.684	57,500	420
32	6.8	14.5	-220	2650	5.108	2.328	52,500	490
42	6.8	14.0	-240	2550	4.345	1.990	40,000	420
52	6.8	13.5	-220	2550	7.920	3.610	72,500	490

TABLE C-3. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 4/9/84

Sample Number	pH (s.u.)	Temp. (^oC)	ORP (mv)	Conductivity (uMHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	7.4		+20	2600	0.1073	0.0440	425	319
21	7.4		+20	2600	0.1064	0.0442	430	314
31	7.4		+20	2650	0.1061	0.0424	425	319
41	7.4		+20	2650	0.1011	0.0436	410	319
51	7.4		+20	2650	0.1016	0.0415	415	308
12	7.0		-180	2900	3.689	1.772	30,000	448
22	7.0		-150	2800	2.308	1.125	19,000	532
32	7.1		-180	2850	3.033	1.440	26,500	266
42	6.9		-180	2750	4.330	2.061	40,000	462
52	7.0		-180	2750	3.235	1.524	28,500	434

TABLE C-4. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 5/7/84

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	7.3	25.0	+90	2600	880 mg/1	550 mg/1	480	269
21	7.3	25.0	+90	2600	860 mg/1	400 mg/1	480	274
31	7.4	25.0	+90	2520	900 mg/1	450 mg/1	490	274
41	7.3	25.0	+90	2650	870 mg/1	520 mg/1	450	286
51	7.3	25.0	+90	2650	830 mg/1	450 mg/1	450	263
12A	6.8	26.0	-170	3200	7.480 %	3.303 %	24,900	*[a]
12B	6.7	24.0	-170	3100	9.108 %	4.013 %	85,000	*
22A	6.6	25.0	-160	3400	9.103 %	4.030 %	99,100	*
22B	6.6	25.0	-170	3250	9.632 %	4.268 %	86,700	*
32A	6.7	25.0	-170	3300	8.190 %	3.621 %	96,500	*
32B	6.9	25.0	-170	3300	6.957 %	3.113 %	66,000	*
42A	6.7	25.0	-170	3350	7.148 %	3.148 %	58,500	*
42B	6.8	25.0	-170	-	11.319 %	5.051 %	137,000	*
52A	6.8	25.0	-170	3200	8.076 %	3.610 %	80,000	*
52B	6.8	25.0	-170	3100	8.117 %	3.627 %	74,100	*
4" column	7.0	25.0	-170	3250	1.857 %	0.803 %	-	-

[a] Samples being reanalyzed due to analytical problems.

TABLE C-5. NEW ORLEANS SLUDGE ABIOTIC DATA FOR 6/18/84

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	7.4	25.0	+130	2400	0.073	0.035	325	246
21	7.4	25.0	+130	2400	0.072	0.033	300	246
31	7.4	25.0	+130	2400	0.069	0.033	310	246
41	7.4	25.0	+130	2400	0.073	0.041	355	246
51	7.4	25.0	+130	2400	0.053	0.030	320	246
12	6.7	25.0	-175	2800	8.08	3.56	84,000	280
22	6.7	25.0	-180	2800	8.31	3.70	60,000	350
32	6.7	25.0	-180	2800	7.44	3.23	76,000	280
42	6.7	25.0	-180	2800	5.32	2.50	60,000	350
52	6.7	25.0	-180	2800	5.32	2.40	68,000	350

TABLE C-6. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 8/6/84

Sample Number	pH (s.u.)	Temp. (°C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11[a]	7.7	26.0	+50	1350	0.60	0.29	110	138
21	7.9	26.0	+50	1350	0.56	0.29	145	138
31	7.9	26.0	+50	1350	0.54	0.27	110	136
41	8.0	26.0	+50	1350	1.08	0.21	130	134
51	8.1	26.0	+50	1350	0.51	0.26	150	134
12A[b]	7.2	26.0	-75	1650	4.10	1.95	25,000	257
12B[b]	7.4	26.0	-100	1650	2.25	1.01	10,000	170
22A	7.2	26.0	-125	1700	8.60	3.97	90,000	391
22B	7.3	26.0	- 95	1700	8.58	3.64	63,000	535
32A	7.3	26.0	-125	1720	7.00	3.13	43,000	378
32B	7.3	26.0	-110	1720	6.60	2.96	60,000	340
42A	7.2	26.0	-130	1700	10.41	4.67	65,000	411
42B	7.2	26.0	-100	1700	5.04	2.27	50,000	374
52A	7.4	26.0	- 50	1500	6.50	2.97	56,000	527
52B	7.3	26.0	-110	1500	4.66	2.16	40,000	434
4"R/C	7.1	26.0	- 90	1500	3.96	1.87	5,000	-

[a] Sample 11: dissolved oxygen = 2.1 ppm

[b] A and B samples were taken from the same location in the lagoon.

TABLE C-7. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 11/14/84

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	8.0	18.0	+223.3	1000	0.05	0.01	190	780
21	8.2	18.0	+223.4	1000	0.05	0.02	175	800
31	8.0	18.0	+219.8	1000	0.05	0.02	170	760
41	8.2	18.0	+221.7	1000	0.02	0.01	180	760
51	8.0	18.0	+219.3	1000	0.003	0.002	180	730
12A[a]	7.1	14.0	-125	2100	6.5	3.8	175,000	4300
12B[a]					4.4	2.6	46,000	
22A	6.9	14.0	-125.0	2100	9.3	5.5	95,000	3600
22B					9.2	5.4	110,000	
32A	7.1	14.0	-125.0	2100	11.2	6.8	104,000	4500
32B					3.9	2.2	80,000	
42A	7.1	14.0	-124.0	2100	10.4	6.3	132,000	4800
42B					5.2	3.0	100,000	
52A	7.1	14.0	-127.0	2100	8.4	5.0	110,000	4600
52B					8.5	5.1	100,000	
4"PVC	7.5	14.0	- 37.0	1400	2.9	1.7	140	4000

[a] A and B samples were taken from same location in the lagoon.

TABLE C-8. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 2/11/85

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-
41	-	-	-	-	-	-	-	-
51	-	-	-	-	-	-	-	-
12A[a]	6.7	12.0	- 25	900	7.98	4.70	70,000	525
12B[a]	-	-	-	-	8.70	5.08	75,000	500
22A	6.8	11.0	- 50	850	10.00	5.91	100,000	700
22B	-	-	-	-	11.26	6.61	80,000	420
32A	6.8	11.0	- 79	1100	8.82	5.18	90,000	530
32B	-	-	-	-	10.27	6.11	85,000	525
42A	6.8	11.0	- 25	900	8.02	4.66	80,000	580
42B	-	-	-	-	11.37	6.63	115,000	600
52A	6.8	11.5	- 25	800	10.14	5.98	100,000	425
52B	-	-	-	-	11.43	6.76	110,000	410
4"PVC	7.2	-	-140	-	0.83	0.74	15,000	340

[a] A and B samples were taken from same location in the lagoon.

TABLE C-9. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 5/14/85

Sample Number	pH (s.u.)	Temp. (°C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	8.1	25.0	+300	600	0.02	0.02	240	200
21	7.9	24.0	+300	600	0.08	0.08	225	210
31	7.7	24.5	+300	610	0.02	0.02	280	220
41	7.6	24.0	+300	650	0.08	0.08	275	230
51	7.6	24.0	+300	610	0.10	0.1	950	250
12A[a]	6.6	23.0	- 50	1500	11.8	7.0	124,000	3200
12B[a]	-	-	-	-	9.7	6.5	80,000	3000
22A	6.9	24.0	- 30	1550	11.5	6.9	85,000	5800
22B	-	-	-	-	8.6	5.0	80,000	4700
32A	6.7	25.0	- 45	1530	11.3	6.6	110,000	3600
32B	-	-	-	-	11.9	7.2	140,000	3900
42A	7.0	22.0	- 65	1650	9.8	5.8	170,000	5400
42B	-	-	-	-	9.0	5.2	124,000	5200
52A	6.7	22.0	- 35	1650	8.9	5.2	84,000	2800
52B	-	-	-	-	13.1	7.6	48,000	1200
4"PVC	7.5	35.5	+100	950	3.4	2.0	25,000	1800

[a] A and B samples were taken from same location in the lagoon.

TABLE C-10. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 8/13/85

Sample Number	pH (s.u.)	Temp. (^{O}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)	TKN (mg/L)
1-2A[a]	7.0	27	-150	2200	7.12	4.23	$2.4X10^4$	430	2652
1-2B[a]	7.1	27	-160	2200	8.51	5.51	$5.0X10^4$	610	----
2-2A	7.0	26	-170	2000	9.19	7.78	$3.6X10^3$	430	3060
2-2B	6.9	26	-170	2200	1.71	1.02	$3.6X10^3$	260	----
3-2A	6.6	27	-180	2000	10.5	8.42	$3.6X10^4$	280	3162
3-2B	6.7	27	-160	2000	5.12	4.60	$2.3X10^4$	110	----
4-2A	6.8	27	-170	2200	7.41	4.83	$9.0X10^4$	185	2754
4-2B	6.8	27	-190	2200	7.07	4.24	$1.1X10^5$	400	----
5-2A	6.9	27	-170	2000	9.7	6.14	$1.7X10^4$	390	2612
5-2B	6.7	27	-200	2000	5.70	3.41	$9.5X10^4$	390	----
PVC	7.2	27	-150	----	7.23	4.37	$3.4X10^4$	320	----

[a] A and B samples were taken from the same location in the lagoon.

TABLE C-11. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 12/19/85

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)	TKN (mg/L)
1-1	6.6	17	+170	0.058	0.011	75	20	26.9
2-1	6.6	17	+170	0.063	0.017	75	22	26.9
3-1	6.6	17	+160	0.061	0.008	60	20	26.9
4-1	6.6	17	+150	0.053	0.017	70	22	30.2
5-1	6.6	17	+150	0.052	0.016	70	21	23.5
1-2A[a]	6.8	17	-125	7.34	4.33	$6.5X10^4$	980	$8.8X10^4$
1-2B[a]	6.7	17	-170	7.12	4.20	$6.5X10^4$	580	
2-2A	6.8	17	-170	8.49	5.03	$7.0X10^4$	900	$8.2X10^4$
2-2B	6.9	17	-165	12.14	7.48	$1.1X10^5$	500	
3-2A	6.8	17	-140	6.45	3.80	$5.2X10^4$	680	$6.1X10^4$
3-2B	6.8	17	-140	6.96	4.13	$5.2X10^4$	490	
4-2A	6.8	17	-170	8.53	5.04	$7.0X10^4$	940	$5.6X10^4$
4-2B	6.7	17	-130	6.09	3.61	$5.0X10^4$	410	
5-2A	6.8	17	-140	8.28	4.95	$7.8X10^4$	800	$8.0X10^4$
5-2B	6.8	17	-140	9.63	5.76	$8.5X10^4$	740	
PVC	7.0	17	-100	0.64	0.39	$4.0X10^3$	9.0	

[a] A and B samples were taken from same location in the lagoon.

TABLE C-12. NEW ORLEANS SLUDGE LAGOON ABIOTIC DATA FOR 3/01/86

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)	TKN (mg/L)
1-1	6.8	16	+225	335	0.033	0.021	220	16	594
2-1	6.8	16	+250	330	0.032	0.019	175	14	613
3-1	6.8	16	+240	328	0.031	0.021	170	14	632
4-1	6.7	16	+240	330	0.029	0.021	125	14.5	672
5-1	6.7	16	+240	332	0.031	0.021	170	14.5	677
1-2A[a]	6.9	16	-250	2500	12.34	7.70	$1.2X10^{5}$	490	$2.9X10^{4}$
1-2B[a]	6.8	16	-220	2400	8.42	5.02	$8.7X10^{4}$		
2-2A	7.0	16	-220	3600	11.31	6.93	$9.0X10^{5}$	380	$2.6X10^{4}$
2-2B	6.7	16	-220	1900	6.10	3.65	$5.5X10^{5}$		
3-2A	6.8	16	-250	2750	7.82	4.74	$8.6X10^{5}$	730	$2.2X10^{4}$
3-2B	6.8	16	-240	2905	8.78	5.38	$9.4X10^{5}$		
4-2A	7.0	16	-240	1780	13.28	8.27	$1.0X10^{5}$	510	$2.8X10^{4}$
4-2B	6.8	16	-240	2600	9.90	6.14	$1.4X10^{5}$		
5-2A	7.0	16	-230	2420	7.39	4.46	$7.8X10^{4}$	520	$3.4X10^{4}$
5-2B	7.0	16	-230	3500	12.78	7.93	$1.1X10^{5}$		
PVC	7.4	17	+ 60	650	0.28	0.10	$4.0X10^{3}$	8.5	

[a] A and B samples were taken from the same location in the lagoon.

TABLE C-13. ABIOTIC DATA ON THE ANAEROBIC SLUDGES BEFORE DISPOSAL TO PORT ARANSAS LAGOON FOR 2/24/84

Sample No.	Temp (°C)	Conductivity (umHOS)	pH (S.U.)	ORP (MV)	Total Solids (g/L)	Volatile Solids (g/L)	% Volatile of total Solid	COD (mg/L)	NH_3 (mg/L)
11	19.0	1000	6.6	-325	17.4	10.3	59	153,000	392
12	19.5	2050	6.6	-350	15.8	9.1	58	148,500	392
21	19.0	800	6.8	-350	18.4	11.1	60	148,500	336
22	19.0	950	6.5	-350	13.7	7.6	56	117,500	392
31	19.0	850	6.6	-350	17.2	10.1	59	157,000	336
32	19.0	900	6.5	-325	13.6	7.7	57	130,000	392
41	19.5	620	6.6	-350	19.6	11.7	60	144,000	448
42	19.0	620	6.5	-350	8.0	3.9	48	99,000	448
51	19.0	-	6.6	-350	19.0	11.3	60	126,000	392
52	19.0	-	6.5	-325	7.8	3.9	50	81,000	392

TABLE C-14. PORT ARANASAS SLUDGE LAGOON ABIOTIC DATA FOR 4/3/84

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	6.6	21.8	-275	1530	0.3491	0.079	1320	252
21	6.6	21.0	-275	1820	0.4282	0.1220	2530	263
31	6.6	20.2	-260	1720	0.3674	0.0865	1830	263
41	6.7	19.9	-270	1530	0.5101	0.1612	3740	252
51	6.6	20.0	-270	1610	0.3815	0.0791	1650	269
12	6.6	20.2	-280	1300	8.886	4.704	129,860	364
22	6.6	20.0	-270	1630	9.949	5.298	184,220	448
32	6.6	19.9	-270	1450	8.258	4.353	135,900	364
42	6.6	20.0	-270	1270	9.721	5.123	143,450	392
52	6.7	20.1	-270	1370	11.077	5.840	208,380	448

TABLE C-15. PORT ARANSAS SLUDGE LAGOON ABIOTIC DATA FOR 5/1/84

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids (mg/L)	Volatile Solids (mg/L)	COD (mg/L)	NH_3 (mg/L)
11	6.8	24.2	-310	2080	361	126	3000	168
21	6.8	24.1	-310	2510	334	109	2900	190
31	6.8	24.1	-310	2250	345	109	2250	202
41	6.8	24.2	-300	2180	259	63	1500	207
51	6.8	24.0	-310	2290	584	265	6200	213
12	6.8	24.1	-320	2180	280	77	1250	196
22	6.8	24.0	-320	2480	429	157	3000	190
32	6.8	24.0	-320	2280	393	135	2600	213
42	6.9	24.1	-300	2190	832	378	6600	196
52	6.9	24.0	-310	2320	295	81	1900	196

TABLE C-16. PORT ARANASAS SLUDGE LAGOON DATA FOR 6/4/84

Sample Number	pH (s.u.)	Temp. (°C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	6.7	25.5	-290	1030	0.25	0.073	600	280
21	6.8	25.8	-280	1030	0.39	0.084	1080	272
31	6.9	25.6	-280	1030	0.22	0.041	570	280
41	6.9	25.8	-280	1030	0.26	0.023	960	269
51	6.9	25.8	-278	1030	0.22	0.033	750	280
12A[a]	7.6	25.5	-320	880	14.09	7.34	154,000	560
12B[a]	7.6	25.5	-320	880	14.11	7.04		
22A	7.6	25.5	-315	880	15.75	7.69	150,000	630
22B	7.6	25.5	-320	880	13.70	7.01		
32A	7.7	25.5	-320	880	16.17	7.47	234,000	630
32B	7.7	25.5	-320	880	14.54	7.43		
42A	7.7	25.5	-320	880	14.21	6.21	108,000	560
42B	7.7	25.5	-320	880	14.22	7.03		
52A	7.6	25.5	-320	880	15.56	7.58	136,000	700
52B	7.6	25.5	-320	880	13.82	7.06		
4"R/C		25.5	-320	880	3.65	0.23	124,000	

[a] A and B samples were taken from same location in the lagoon.

TABLE C-17. PORT ARANSAS SLUDGE LAGOON DATA FOR 7/2/84

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	6.7	29.1	-260	170	0.27	0.069	1350	275
21	6.8	29.0	-260	170	0.22	0.012	880	287
31	6.9	29.0	-260	170	0.23	0.058	950	286
41	6.9	29.1	-260	170	0.21	0.070	1050	280
51	6.9	29.0	-260	170	0.21	0.056	725	287
12	7.6	29.0	-300	110	15.67	8.01	135,000	210
22	7.6	28.9	-300	110	15.87	7.62	128,000	210
32	7.6	28.9	-300	110	16.57	8.27	126,000	490
42	7.6	28.9	-300	110	17.02	7.48	150,000	840
52	7.7	29.0	-300	110	16.48	8.41	118,000	350

TABLE C-18. PORT ARANSAS SLUDGE LAGOON ABIOTIC DATA FOR 1/15/85

Sample Number	pH (s.u.)	Temp. (^{o}C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)
11	8.3	9.0	-180	160	0.23	0.19	825	72
21	8.3	9.2	-180	149	0.24	0.19	950	74
31	8.3	9.0	-180	149	0.21	0.16	1100	75
41	8.3	9.2	-170	150	0.23	0.13	950	72
51	8.2	9.0	-170	110	0.20	0.13	875	72
12A[a]	6.9	10.0	-275	170	10.59	5.37	70,000	380
12B[a]	6.9	----	-270	---	9.97	5.27	75,000	400
22A	6.8	9.5	-280	150	15.46	7.63	155,000	420
32A	6.8	9.9	-270	172	13.83	7.28	210,000	500
32B	6.9	----	-265	---	14.52	7.68	130,000	440
42B	6.8	----	-280	---	13.36	6.99	200,000	500
52A	6.9	9.0	-275	122	13.87	7.07	110,000	490
52B	6.9	----	-285	---	14.48	7.56	110,000	510
4"PVC	7.5	9.5	-260	150	1.23	0.71	50	460

[a] A and B samples were taken from same location in the lagoon.

TABLE C-19. PORT ARANSAS SLUDGE LAGOON ABIOTIC DATA FOR 5/17/85

Sample Number	pH (s.u.)	Temp. (°C)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)	TKN (mg/L)
1-1	7.7	26.0	+ 50	119	0.21	0.16	860	14.0	93.8
2-1	7.5	26.2	+ 45	102	0.19	0.16	540	13.5	20.4
3-1	7.4	26.1	+ 89	88	0.19	0.15	600	13.5	54.1
4-1	7.6	26.0	+ 40	85	0.22	0.17	970	15.0	102.0
5-1	7.4	26.1	+ 48	60	0.19	0.16	640	14.0	71.4
1-2A[a]	6.9	25.9	-255	129	4.08	2.08	68,000	76	1326
1-2B[a]	---	----	----	---	5.45	2.64	---	80	---
2-2A	6.8	26.1	-270	102	3.89	1.19	55,000	130	489.6
2-2B	---	----	----	---	11.2	5.80	---	230	---
3-2A	6.9	26.1	-285	91	7.08	3.52	44,000	140	132
3-2B	---	----	----	---	8.17	4.06	---	144	---
4-2A	6.8	26.1	-245	90	2.38	1.20	48,000	81	102
4-2B	---	----	----	---	1.38	0.73	---	108	---
5-2A	6.8	26.2	-255	75	9.92	4.74	40,000	188	102
5-2B	---	----	----	---	6.75	3.43	---	220	---
TX PVC	7.8	31.0	-300	119	0.56	0.40	44,000	---	---

[a] A and B samples were taken from the same location in the lagoon.

TABLE C-20. PORT ARANSAS LAGOON ABIOTIC DATA FOR 2/18/86

Sample Number	pH (s.u.)	Temp. (^oC)	ORP (mv)	Conductivity (umHOS)	Total Solids %	Volatile Solids %	COD (mg/L)	NH_3 (mg/L)	TKN (mg/L)
1-1	7.4	14	+ 80	60	0.183	0.074	520	7.5	<5
2-1	7.3	16	+ 80	55	0.184	0.072	550	8.0	<5
3-1	7.3	15	+ 85	50	0.179	0.086	490	6.2	8.4
4-1	7.7	15	+ 85	49	0.167	0.051	275	5.3	<5
5-1	7.5	15	+ 85	50	0.194	0.067	275	6.5	7.0
1-2A[a]	7.3	14	+ 85	53					
1-2B[a]	6.8		-180	2200	6.29	3.00	$7.5X10^4$	82	8928
2-2A	7.2	14	+ 85	50					
2-2B	6.7		-210	2050	8.76	4.34	$9.6X10^4$	111	LOST
3-2A	7.1	14	+ 85	51					
3-2B	6.7		-205	2050	7.81	3.90	$5.6X10^4$	103	10098
4-2A	7.3	14	+ 80	49					
4-2B	6.7		-205	2050	7.97	3.72	$9.0X10^4$	115	2839
5-2A	7.2	14	+ 85	49					
5-2B	6.8		-200	2010	7.54	3.70	$5.6X10^4$	105	2188
PVC					0.69	0.25	$1.2X10^3$	550	2188

[a] A and B samples were taken from the same location in the lagoon. In this sampling, "bottom water" and "sediment" samples were taken in error; some abiotic measurements were made on the bottom water samples, but they were largely disregarded with respect to pathogen enumeration.

TABLE C-21. QUALITY ASSURANCE FOR ABIOTIC ANALYSES NEW ORLEANS LAGOON

DATE	SAMPLE	COD mg/L	NH$_3$ mg/L
3/12/84	51	600	298
	51 Dup	580	325
	Mean	590	311.5
	Standard Deviation	±14.1	±19.1
	51 Spike	1120/97% Recovery	498/88% Recovery
4/9/84	11	425	319
	11 Dup	420	314
	Mean	422.5	316.5
	Standard Deviation	±3.5	±3.5
	11 QC	1020/103% Recovery	504/90% Recovery
5/7/84	11	480	269
	11 Dup	430	274
	Mean	455	271.5
	Standard Deviation	±35	±3.5
	11 Spike	900/98% Recovery	470/91% Recovery
6/18/84	51	320	246
	51 Days	310	246
	Mean	315	246
	Standard Deviation	±7.07	0
	51 Spike	900/97.8% Recovery	638/101% Recovery
	52	8,500	280
	52 Days	8,250	380
	Mean	8,375	330
	Standard Deviation	±1.8 x 10^2	±70.7
	52 Spike	22,000/95.6% Recovery	2310/102% Recovery

(Continued)

TABLE C-21 (continued)

DATE	SAMPLE	COD mg/L	NH_3 mg/L
8/6/84	51	150	134
	51 Dup.	125	134
	Mean	137.5	134
	Standard Deviation	±17.7	±0
	51 Spike	790/110% Recovery	525/99% Recovery
	52	40,000	411[a]
	52 Dup.	40,000	374
	Mean	40,000	393
	Standard Deviation	±0	±26.2
	52 Spike	160,000/101% Recovery	765/95% Recovery
11/14/84	51	180	730
	51 Dup.	180	750
	Mean	180	740
	Standard Deviation	±0	±14.1
	51 Spike	700/94% Recovery	759/98% Recovery
	52	550	4600
	52 Dup.	610	4600
	Mean	580	4600
	Standard Deviation	±42.4	±0
	52 Spike	1140/97% Recovery	4630/94% Recovery
1/15/85	51		
	51Q		
	Mean	BOTTOM SAMPLES ONLY	
	Standard Deviation		
	51 Spike		
	52	100,000	525
	52	115,000	500
	Mean	107,500	512.5
	Standard Deviation	± 7,500	±12.5
	52 Spike	215,000/91.5% Recovery	600/98.0% Recovery

(Continued)

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[a] Using ammonia probe in sediment sample - discontinued procedure.

TABLE C-21 (continued)

DATE	SAMPLE	COD mg/L	NH_3 mg/L
5/14/85	51	275	250
	51Q	310	240
	Mean	292.5	245
	Standard Deviation	±24.7	±5.0
	52 Spike/Recovery %	950/117%	360/103%
	52A	420	2800
	52AQ	500	3200
	Mean	460	3000
	Standard Deviation	±56.6	±283.2
	52A Spike/Recovery %	1040/102%	5100/134%
	52B	240	1200
	52BQ	275	1500
	Mean	257	1350
	Standard Deviation	±24.7	±212.1
	52B Spike/Recovery %	700/90%	2900/132%
8/13/85	52A	17000	390
	52AQ	15000	390
	Mean	16000	390
	Standard Deviation	±1414	± 0.0
	52A Spike/Recovery %	$1.54x10^5$/12 3%	410/91.1%
12/19/85	51	70	21
	51Q	75	20
	Mean	72.5	20.5
	Standard Deviation	±3.54	±0.707
	52 Spike/Recovery %	535/104%	32.0/120%
	52A	$7.8x10^4$	800
	52AQ	$7.7x10^4$	790
	Mean	$7.75x10^4$	795
	Standard Deviation	±707	± 7.07
	52A Spike/Recovery %	$1.9x10^5$/114 %	821/110%
	52B	$8.5x10^4$	740
	52BQ	$8.4x10^4$	740
	Mean	$8.45x10^4$	740
	Standard Deviation	±707	±0.00
	52B Spike/Recovery %	$1.7x10^5$/113 %	749/90%

(Continued)

TABLE C-21 (continued)

DATE	SAMPLE	COD mg/L	NH_3 mg/L
03/01/86	51	170	14.0
	51Q	160	14.5
	Mean	165	14.25
	Standard Deviation	±5.0	±0.35
	51 Spike/Recovery %	590/98%	150/107%
	52A	6.8×10^4	520
	52AQ	8.0×10^4	540
	Mean	7.4×10^4	530
	Standard Deviation	8.48×10^3	14.14
	52A Spike/Recovery %	9.4×10^4/77%	600/97%
	52B	1.02×10^5	
	52BQ	1.10×10^5	
	Mean	1.06×10^5	
	Standard Deviation	5.65×10^3	
	52B Spike/Recovery %	1.28×10^5/98 %	

TABLE C-22. QUALITY ASSURANCE FOR ABIOTIC ANALYSES PORT ARANSAS LAGOON

DATE	SAMPLE	COD mg/L	NH_3 mg/L
4/3/84	11	1320	252
	11 Dup	1320	258
	Mean	1320	255
	Standard Deviation	0	4.2
	11 Spike	1280/101% Recovery	437/87% Recovery
5/1/84	11	3000	168
	11 Dup	2800	202
	Mean	2900	185
	Standard Deviation	141	24
	11 Spike	1320/106% Recovery	381/88% Recovery
6/2/84	51	750	280
	51 Days	750	280
	Mean	750	280
	Standard Deviation	0	±0
	51 Spike	1200/104% Recovery	670/101% Recovery
	52	136,000	560
	52 Days	140,000	490
	Mean	138,000	525
	Standard Deviation	$\pm 2.8 \times 10^3$	±49.4
	52 Spike	256,000/98% Recovery	2760/85% Recovery
7/3/84	51	725	275
	51 Dup.	750	280
	Mean	738	278
	Standard Deviation	±17.7	±3.53
	51 Spike	1325/97% Recovery	678/97% Recovery
	52	590	210
	52 Dup.	550	210
	Mean	570	210
	Standard Deviation	±28.3	±0
	52 Spike	1140/97% Recovery	2100/98% Recovery

(Continued)

TABLE C-22 (continued)

DATE	SAMPLE	COD mg/L	NH_3 mg/L
5/17/85	51	350	172
	51Q	350	170
	Mean	350	171
	Standard Deviation	± 0.0	± 1.41
	51 Spike/Recovery %	900/103%	250/92.5%
	52A	90,000	2000
	52AQ	90,000	2100
	Mean	90,000	2050
	Standard Deviation	±0.0	± 70.7
	52A Spike/Recovery %	220,000/113%	2900/104%
	52B	112,000	2800
	52BQ	110,000	2800
	Mean	111,000	2800
	Standard Deviation	±1,414.2	±0.00
	52B Spike/Recovery %	220,000/102%	2900/180%
8/13/85	51	640	14
	51Q	650	15
	Mean	645	14.5
	Standard Deviation	±5.0	±0.5
	51 Spike/Recovery %	740/98.7%	40/85%
	52A	40,000	188
	52AQ	65,000	220
	Mean	52,500	204
	Standard Deviation	±17.678	±22.6
	52A Spike/Recovery %	$1.5X10^5$/94.8%	310/96.9%
02/18/86	51	275	6.5
	51Q	275	7.0
	Mean	275	6.75
	Standard Deviation	±0.00	±0.35
	52 Spike/Recovery %	700/97%	72.5/103%
	52A	$7.8x10^4$	105
	52AQ	$7.7x10^4$	105
	Mean	$7.75x10^4$	105
	Standard Deviation	±707	± 0.00
	52A Spike/Recovery %	$1.9x10^5$/114%	1010/97%

APPENDIX D

TABLE D-1. CLIMATIC DATA TAKEN FROM THE NEW ORLEANS SITE

WEATHER STATION DATA

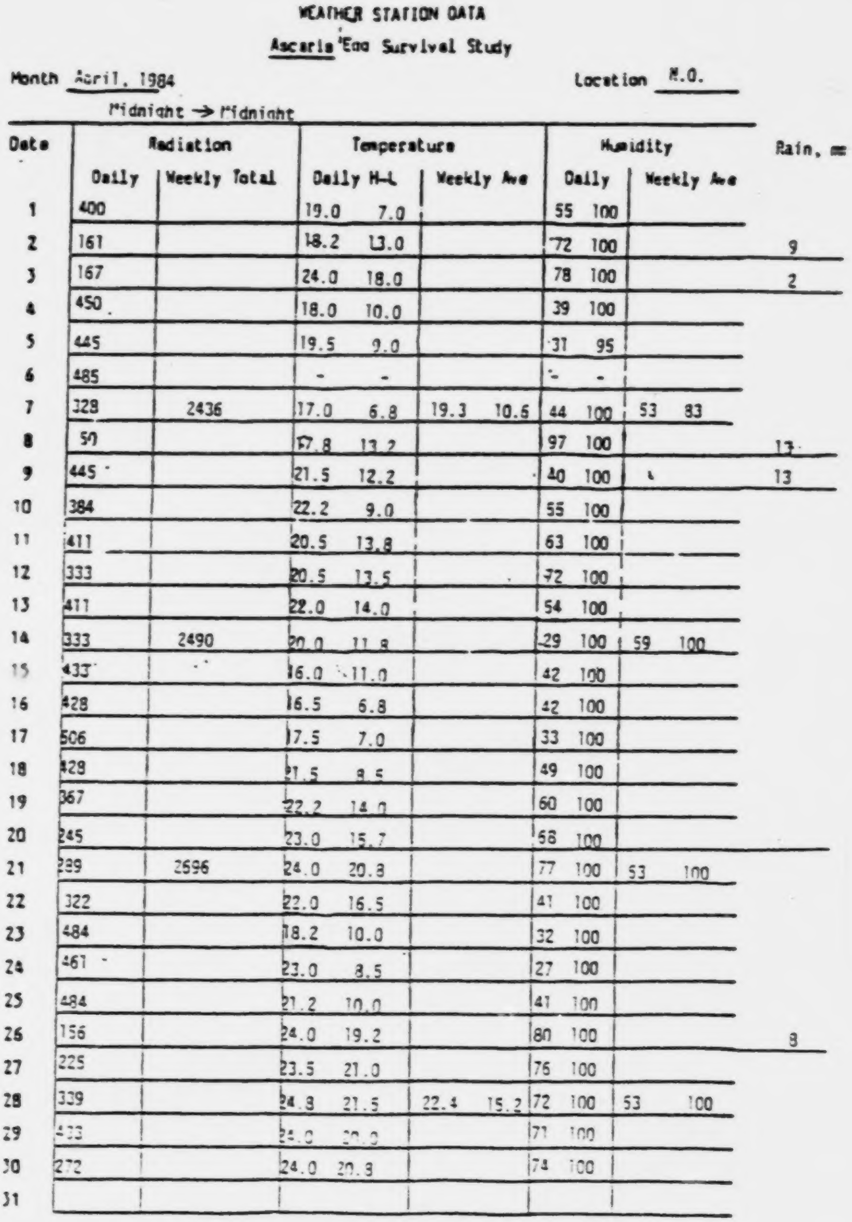
Ascaris Egg Survival Study

Month March 1984 Location New Orleans

Midnight to Midnight

Date	Radiation		Temperature				Humidity				Rain(mm)
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		
							L	H			
1	339		11.7	-2.0			43	100			
2	245		16.0	2.2			57	100			
3	279		19.0	6.5			52	100			
4	239		18.8	11.9			63	100			
5	34		12.0	10.0			98	99			56
6	61		10.0	5.5			59	100			
7	378	1574	11.0	-1.5	14.5	3.7	32	100	56	100	
8	389		17.0	0.2			36	100			
9	400		14.0	4.0			35	100			
10	328		16.0	2.0			54	100			
11	334		17.1	4.5			50	100			
12	133		16.1	8.0			68	100			18
13	322		22.0	11.0			71	100			
14	328	2234	22.0	15.0	17.7	6.4	67	100	55	100	
15	161		21.8	11.0			67	100			
16	356		21.0	12.7			54	100			
17	395		22.5	11.0			53	100			
18	361		21.3	9.8			59	100			
19	245		22.3	14.0			63	100			
20	222		14.8	4.8			55	100			7
21	439	2179	17.8	4.2	20.2	9.6	25	100	54	100	28
22	350		19.0	5.0			43	100			38
23	119		19.8	13.0			33	100			
24	137		22.5	15.0			77	100			
25	434		19.0	9.0			42	100			
26	128		17.8	8.2			65	100			
27	156		24.0	15.5			74	100			
28	356	1952	19.9	12.5	20.1	11.2	50	100	55	100	
29	339		15.5	8.0			40	71			
30	367		16.0	4.0			33	100			
31	361		17.0	6.0			43	100			
Monthly Total	9056		Mean Hi [illegible]		Mean Lo [illegible]		Average		54	99	147

TABLE D-1 (Continued)



WEATHER STATION DATA

Ascaris Egg Survival Study

Month April, 1984 Location N.O.

Midnight → Midnight

Date	Radiation		Temperature				Humidity				Rain, mm
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		
1	400		19.0	7.0			55	100			
2	161		18.2	13.0			72	100			9
3	167		24.0	18.0			78	100			2
4	450		18.0	10.0			39	100			
5	445		19.5	9.0			31	95			
6	485		-	-			-	-			
7	328	2436	17.0	6.8	19.3	10.6	44	100	53	83	
8	50		17.8	13.2			97	100			13
9	445		21.5	12.2			40	100			13
10	384		22.2	9.0			55	100			
11	411		20.5	13.8			63	100			
12	333		20.5	13.5			72	100			
13	411		22.0	14.0			54	100			
14	333	2490	20.0	11.8			29	100	59	100	
15	433		16.0	11.0			42	100			
16	428		16.5	6.8			42	100			
17	506		17.5	7.0			33	100			
18	428		21.5	8.5			49	100			
19	367		22.2	14.0			60	100			
20	245		23.0	15.7			58	100			
21	289	2696	24.0	20.8			77	100	53	100	
22	322		22.0	16.5			41	100			
23	484		18.2	10.0			32	100			
24	461		23.0	8.5			27	100			
25	484		21.2	10.0			41	100			
26	156		24.0	19.2			80	100			8
27	225		23.5	21.0			76	100			
28	339		24.8	21.5	22.4	15.2	72	100	53	100	
29	433		24.0	20.0			71	100			
30	272		24.0	20.8			74	100			
31											

TABLE D-1 (Continued)

Ascaris Egg Survival Study

Month May, 1984 Location N.O.

Date	Radiation		Temperature				Humidity				Rain, mm
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		
1	194		23.2	20.0			80	100			
2	167		22.8	20.2			60	100			
3	189		23.5	21.5			78	100			
4	311		24.5	20.0			42	100			
5	255		24.0	19.2			80	100			
6	311		24.0	23.0			61	100			
7	389	1816	25.0	23.0	23.9	21.0	70	100		100	
8	233		22.5	17.0			62	100			6
9	489		21.0	12.0			34	100			
10	455		22.5	12.0			44	100			
11	489		21.0	12.0			49	100			
12	450		23.5	11.5			46	100			
13	433		24.0	13.0			47	100			
14	433	2982	25.5	15.5	22.9	13.6	49	100	45	100	
15	372		25.2	18.5			59	100			
16	450		23.0	17.2			41	100			
17	372		23.0	17.0			60	100			
18	445		22.0	14.8			51	100			
19	406		22.0	15.5			53	100			
20	111		21.0	18.0			91	100			65
21	300	2456	23.8	18.2	22.9	17.0	77	100	62	100	
22	122		23.0	18.5			88	100			
23	406		23.8	18.0			58	100			
24	367		24.2	18.8			52	100			
25											
26											
27											
28											
29											
30											
31											

Monthly Total ________ Mean Hi ____ Mean Lo ____ Average ________

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Ova Survival Study

Month June 1984 Location T.U.

Date	Radiation		Temperature				Humidity				Rain
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		MM
1	431.03		26.7	10.0			33	87			
2	396.5	827.5	26.7	11.7	26.7	10.8	52	97	43	92	
3	400.8		28.3	12.8			48	93			
4	318.9		29.4	16.1			59	93			
5	387.9		30.0	17.7			55	90			
6	237.1		29.4	22.8			65	87			
7	353.4		30.0	23.9			53	87			
8	392.2		29.4	21.7			53	90			
9	293.1	2383.4	30.6	21.1	29.5	19.4	53	90	55	90	0.25
10	318.9		30.6	21.1			57	90			
11	288.8		29.4	20.0			67	90			36
12	293.1		29.4	19.4			63	97			62
13	362.1		30.0	19.4			55	93			
14	344.8		32.2	19.4			37	97			
15	271.5		32.2	21.1			55	90			11
16	202.6		28.9	22.8	30.4	20.5	67	87			18
17	297.4		28.9	20.6			61	94			
18	211.2		28.9	21.1			69	94			7
19	383.6		33.8	20.0			52	97			
20	366.4		34.4	23.9			51	90			
21	340.5		33.3	23.9			54	91			
22	318.9		33.8	25.0			54	91			
23	254.3	2172.3	32.3	24.4	32.3	22.7	61	91	65	93	
24	68.96		30.6	19.2			77	94			27
25	396.5		33.0	19.2			65	100			
26	444.6		31.2	19.2			53	100			
27	277.9		29.8	21.2			73	100			14
28	166.7		26.0	21.0			92	100			2
29	277.9		27.5	23.0			69	100			
30	166.7	1799.3	27.0	20.5	29.3	23.5	93	100			16
31											

Monthly Total 9254.2 Mean Hi 29.5 Mean Lo 19.4 Average 53 93 193mm

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Ova Survival Study

Month July 1984 Location T.U.

Date	Radiation		Temperature				Humidity				Rain
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		MM
1	188.9		27.0	20.0			74	100			
2	283.4		32.5	21.0			63	100			8
3	389.0	1750.5	31.0	22.2	28.7	21.3	64	100	75.4	100	
4	222.3		31.2	22.0			62	100			
5	261.2		31.0	22.2			60	100			
6	327.9		30.5	21.0			62	100			
7	272.3		30.5	22.5			70	100			
8	355.7		30.5	23.2			68	100			5
9	294.5		32.0	22.5			64	100			
10	400.0	2133.9	30.5	22.2	30.9	22.2	59	100	63.6	100	3
11	389.0		32.5	23.2			65	100			
12	266.8		30.5	23.5			65	100			
13	311.2		29.0	22.5			68	100			
14	416.8		29.2	22.2			66	100			13
15	266.8		29.0	23.0			61	100			
16	105.6		29.5	23.0			74	100			
17	222.3	1978.5	29.2	23.0	29.8	22.9	71	100			10
18	194.5		29.0	23.8			78	100			23
19	366.8		29.8	22.5			63	100			
20	361.2		28.5	22.2			50	100			
21	355.7		32.0	21.8			54	100			
22	361.2		32.0	23.5			58	100			
23	166.7		29.2	22.0			64	100			22
24	111.2	1917.3	28.5	22.5	29.9	22.6	76	100	63.3	100	7
25	255.6		26.8	22.5			83	100			23
26	433.5		29.8	22.5			61	100			24
27	250.1		29.0	22.0			68	100			6
28	305.7		28.0	22.0			72	100			11
29	233.4		27.5	23.0			70	100			
30	250.0		28.2	23.0			74	100			
31	205.6	1933.9	27.5	21.5	28.1	22.4	75	100	73.3	100	
Monthly Total		3435.9	Mean Hi 29.3		Mean Lo 22.4		Average 66.3/100				155

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Ova Survival Study

Month August 1984 Location T.U.

Date	Radiation		Temperature				Humidity			Rain
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave	MM
1	161.2		26.5	22.5			75	100		11
2	94.5		25.0	20.2			93	100		47
3	33.3		26.0	24.0			94	100		2
4	83.4		28.5	22.8			73	100		
5	227.9		29.5	23.8			75	100		26
6	138.9		27.2	23.0			85	100		
7	300.1	1039.3	29.8	22.5	27.5	22.7	61	100	79.4	13
8	311.2		30.0	22.0			56	100		
9	333.5		31.2	22.2			60	100		
10	*		31.2	22.8			59	100		
11	*		29.0	23.8			70	100		6
12	*		29.0	24.0			64	100		
13	79.0		29.0	23.0			72	100		28
14	166.7		28.0	23.0	29.6	23.0	73	100	64.9	
15	333.5		30.0	22.5			64	100		
16	277.9		31.0	23.5			70	100		
17	188.9		31.2	24.5			66	100		
18	266.8		31.5	24.5			70	100		
19	250.1		31.8	23.8			65	100		
20	200.1		29.8	24.5			55	100		
21	222.3	1739.6	30.8	24.5	30.3	24.0	57	100	63.9	
22	166.7		29.5	23.5			72	100		3
23	122.3		30.0	24.5			61	100		3
24	294.5		30.2	23.5			51	100		
25	127.8		30.0	23.8			68	100		15
26	233.4		29.2	24.0			63	100		
27										
28										
29										
30										
31										

Monthly Total ______ Mean Hi ____ Mean Lo ____ Average ______

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Egg Survival Study

Month September 1984 Location New Orleans
Midnight to Midnight

Date	Radiation		Temperature				Humidity				
	Daily	Weekly Total	Daily H-L		Weekly	Ave	Daily		Weekly	Ave	Rain (mm)
1	405.7		30	22			100	51			
2	300.1		30	22			100	53			
3	311.2	2100.8	30	23	25.7	23.8	99	51	100	60	2.0
4	111.2		25	22			99	86			
5	400.1		27	22			100	45			
6	339.0		29	21			100	62			
7	200.3		29	20			100	69			
8	216.7		29	23			100	67			
9	322.3		30	22			97	63			
10	400.1	2411.2	33	21	28.9	21.9	96	52	93.7	62.0	
11	316.8		31	20			97	57			
12	377.9		32	21			95	57			
13	361.2		33	22			95	53			
14	294.5		32	22			95	53			
15	272.3		32	22			95	54			
16	333.0		29.5	22			96	55			
17	222.3	2178.4	27	23	30.8	21.0	95	58	95.4	56.3	
18	283.4		29	22			94	65			
19	261.2		28	22			96	65			
20	289.0		26	23			100	68			
21	111.2		29	23			96	68			31.0
22	272.3		30	23			96	66			
23	272.3		31	20			97	65			
24	378.3	1861.9	29	19	28.4	27.3	96	55	96.7	65.8	
25	272.3		30	19			96	46			
26	333.5		30	18			95	48			
27	311.2		30	18			95	53			
29	311.2		28	19			94	51			
29	366.8		24	16			95	34			
30	366.8	1961.8	24	15	27.2	17.5	97	39	92.0	46.5	
31											

Monthly Total 11,014.10 Mean Hi 29.2 Mean Lo 20.8 Average 96.7 57.2 33.0

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Egg Survival Study

Month October 1984 Location New Orleans

Midnight to Midnight

Date	Radiation		Temperature				Humidity				
	Daily	Weekly Total	Daily H-L		Weekly	Ave	Daily		Weekly	Ave	Rain (mm)
1	333.5	2295.3	23	09	26.4	16.3	97	35	92.7	44.9	
2	327.9		23	09			98	35			
3	327.9		24	08			98	50			
4	326.8		24	11			96	56			
5	262.2		29	16			98	60			
6	257.1		28	20			97	57			
7	194.5		29	19			98	62			
8	54.0	1732.4	26	19	26.3	14.6	98	62	97.4	54.6	
9	135.9		25	18			98	63			8
10	263.4		27	19			97	68			10
11	261.2		27	17			97	53			
12	272.3		28	17			96	52			
13	283.4		27	17			96	53			
14	194.5		24	18			96	53			23
15	203.6	1634.3	24	19	27.0	17.4	96	55	96.5	64.3	
16	290.9		24	23			97	77			
17	53.6		28	21			96	55			8
18	239.0		27	23			97	77			
19	271.9		28	21			95	79			
20	283.4		28	21			97	77			
21	205.6		27	24			97	77			3
22	301.2	1640.6	28	23	27.6	22.3	97	55	96.7	70.7	
23	144.5		29	20			95	70			
24	261.2		27	18.5			95	59			
25	283.6		28	18.5			96	53			
26	253.6		27.5	17			99	54			
27	*		28.5	19			96	53			
28	*		28	20			97	51			
29	161.2	1175.9	28	20	27.6	19.0	96	61	96.1	58.0	
30	161.2		27	20			99	75			
31	222.3		28	20			98	65			

Monthly Total 8,978.5 Mean Hi 20.4 Mean Lo 18.2 Average 97.6 59.3 52

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Egg Survival Study

Location New Orleans

Month November 1984

Midnight to Midnight

Date	Radiation		Temperature				Humidity				Rain (mm)
	Daily	Weekly Total	Daily H-L		Weekly	Ave	Daily		Weekly	Ave	
1	222.3		28	20			98	68			
2	72.2		21	16			99	81			
3	83.4		18	16			100	81			
4	239.0		23	12			100	65			
5	230.7	1231.1	33	17	25.0	17.3	93	37	98.1	65.4	
6	240.5		21	08			96	27			
7	224.3		21.5	07			97	41			
8	189.5		24	10.5			97	53			
9	65.0		23.5	13			98	59			
10	247.0		21.5	15			97	47			
11	269.8		15	10			96	47			
12	177.8	1413.9	15	10	10.4	10.5	96	43	97.5	44.9	
13	283.4		17	11			96	43			
14	244.5		24	12			96	45			
15	244.5		24	10			97	43			
16	205.5		23	13			96	62			
17	250.1		22	10			98	59			
18	172.3		25	14			99	63			18
19	177.9	1578.3	23	13	22.6	12.6	90	57	96.8	52.8	
20	105.6		13	06			100	53			
21	261.2		14	06			89	52			
22	311.2		15	04			99	32			
23	255.6		14	02			100	34			
24	238.9		17.5	01			100	31			
25	194.5		23	06			99	57			
26	61.6	1428.6	22	18.5	16.9	6.2	93	65	97.1	47.4	10
27	55.6		21	11			100	68			
28	261.2		14	03			98	37			
29	216.7		18	00			99	46			
30	116.7		23	09			100	85			
31											

Monthly Total 5,651.9 Mean Hi 20.6 Mean Lo 10.1 Average 97.1 52.6 28

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Egg Survival Study

Month December 1984 Location New Orleans

Midnight to Midnight

Date	Radiation		Temperature				Humidity				
	Daily	Weekly Total	Daily	H-L	Weekly	Ave	Daily		Weekly	Ave	Rain (mm)
1	44.5		17	7			90	75			
2	172.3		16	10			93	67			
3	22.3	889.3	15	6	17.7	6.6	97	43	96.7	59.4	
4	19.2		11	6			97	95			25
5	18.7		11	5			96	44			3
6	22.4		10	-3			93	19			
7	189.6		16.5	-3			96	33			
8	311.3		19.5	5			95	53			
9	264.4		20	9.5			98	49			
10	71.5	591.5	18	13	15.4	4.6	100	61	96.9	50.2	
11	121.3		22	13			97	57			
12	195.9		24	14.5			96	53			
13	171.1		24	18			96	55			
14	146.2		24.5	13.5			97	63			
15	167.9		14	15.5			97	54			
16	102.6		24.5	15			94	58			
17	55.6	885.6	26	12.5	24	14.6	95	67	97.1	57.7	
18	112.3		21	16.5			97	69			
19	194.5		22.5	15.5			97	62			
20	161.7		23	15			97	63			
21	62.2		24	16.5			89	61			
22	58.1		22	14			93	66			1
23	87.1		23.5	14.5			99	59			
24	164.8	851.7	25	15.5	23.0	15.4	97	63	96.9	63.3	
25	96.4		19	12			97	68			
26	21.8		19	11			90	68			
27	31.1		25.5	18			90	71			
28	167.9		27	16			90	54			
29	189.4		27	17			90	67			
30	146.2		27	18			93	71			
31	99.5	743.3	25	17	24.2	15.6	90	74	91.4	67.6	19
Monthly Total	3,664.2		Mean Hi	23.9	Mean Lo	11.4	Average		95.9	59.6	48

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Egg Survival Study

Month January 1985 Location New Orleans
Midnight to Midnight

Date	Radiation		Temperature				Humidity				
	Daily	Weekly Total	Daily	H-L	Weekly	Ave	Daily		Weekly	Ave	Rain (mm)
1	37.4		21	12			72	93			15
2	48.6		12	6			57	94			6
3	42.7		6	0			47	68			4
4	307.2		8	-2			34	64			
5	291.8		11	-2			37	69			
6	139.2		14	-1			21	74			
7	252.1	1116.0	27.5	2	14.2	2.1	23	94	42.3	80.5	
8	272.3		15.5	1			40	98			
9	239.0		27	6.5			54	99			
10	253		22	8			64	93			
11	239.0		12	2			49	87			
12	172.8		6.5	3			21	23			
13	61.1		6.0	3			57.5	94			4
14	171.0	1272.6	17.5	-3	12.8	3.4	69	44	52.3	92	
15	255.7		17	1			36	94			
16	122.3		13	2			53	97.5			
17	77.8		13	0			53	97.5			16
18	183.4		15.5	3			39	97			16
19	233.4		17	-10			39	97			5
20	150.1		-3	-10			38	95			1
21	152.0	1172.8	5	-8	10.1	-3.3	33	97	44.0	96.6	
22	225.6		15	-3			55	92			
23	116.7		9	6			40	98			
24	72.3		13	4			69	98			4
25	230.1		18.5	1.5			30	100			
26	305.7		10	-1			26	93			
27	116.7		18	7			68	98			7
28	190.0	1167.1	8	1	13.1	2.2	72	98	47.1	96.2	11
29	261.2		18	8			61	95			
30	194.5		23	18			69	97			
31	116.7		22	4			79	97			

Monthly Total 5300.9 Mean Hi 12.6 Mean Lo 1.2 Average 47.7 91.4 89

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Egg Survival Study

Month February 1985 Location New Orleans

Midnight to Midnight

Date	Radiation			Temperature				Humidity				
	Daily	Weekly	Total	Daily	H-L	Weekly	Ave	Daily		Weekly	Ave	Rain (mm)
1	89.9			7	-1			77	97			
2	150.1			2	-1			65	98			
3	216.7			7.5	3			45	83			
4	172.3	1200.4		17.5	8	13.9	5.6	67	99	66.1	95.1	4
5	55.6			18	5.5			96	99			17
6	217.9			12	3.0			62	99			29
7	312.2			12	2.0			37	97			16
8	310.0			12	0.5			24	92			
9	355.8			18	7.5			55	96			
10	319.1			19	11			52	96			9
11	153.4	1706.2		9	3	14.3	4.6	42	95	52.5	96.6	
12	416.8			14.5	-2			22	90			
13	433.5			19.0	2.5			12	97			
14	217.9			14	-0.5			31	95			
15	257.1			14	-2			30	97			
16	344.8			19	7.5			39	97			
17	111.1			22.5	12			65	93			
18	53.4	3178.5		21	12	17	4.2	70	94	39.9	96.3	
19	172.3			19	9			71	99			
20	333.5			20	14.5			91	99			
21	217.9			21	16			69	99			
22	222.3			23	18.5			70	99			
23	183.4			24	18			67	97			
24	105.6			19	15			72	98			23
25	102.0	1428.4		18	15	20.6	15.1	86	99	69.4	97.6	13
26	94.5			19	14			77	100			
27	72.3			18	12.5			68	99			
28	305.7			18.5	14			52	91			
29												
30												
31												

Monthly Total 6313.7 Mean Hi 16.5 Mean Lo 7.4 Average 52.2 96.3 113

TABLE D-1 (Continued)

WEATHER STATION DATA

***Ascaris* Egg Survival Study**

Month March 1985 Location New Orleans

Midnight to Midnight

Date	Radiation		Temperature				Humidity				Rain(mm)
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		
1	111.2		21	16.5			100	83			
2	239.0		22.5	12			98	61			
3	227.9		22.0	19			100	73			
4	211.2	1261.8	26	20.5	21	15.5	100	64	98.3	68.3	
5	277.9		22	14			98	49			
6	505.7		21	11.5			79	23			
7	377.9		24	8.5			98	63			
8	350.1		27	18			97	58			
9	305.7		28	19			100	47			
10	239.0		27.5	17.5			99	60			
11	238.9	2295.2	26.5	15	25.1	14.8	97	52	95.4	50.3	
12	250.0		27	16			98	60			
13	283.4		27	15.5			97	49			
14	266.7		28	13.5			99	41			15
15	333.4		19	13.5			93	46			
16	77.8		27	13.5			99	92			
17	333.4		20.5	10			96	33			
18	239.0	1783.7	18	2	23.8	12	92	28	96.3	49.9	15
19	355.6		20	4			96	36			
20	116.7		19	15			99	69			
21	444.6		22	10			98	45			
22	239.0		19	8			95	49			
23	333.4		23	14			99	49			
24	466.8		26	11			98	27			
25	355.6	2311.7	26	11	22	10.4	96	35	97.3	44.3	73
26	361.2		24	17			99	54			
27	100.03		25	20			99	61			14
28	322.3		25	16			98	64			
29	239.0		26	20			98	67			
30	216.7		26	14			98	69			
31	244.5		18	6			98	43			
Monthly Total	8664.2		Mean Hi 23.36		Mean Lo 13.3		Average 59				

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Egg Survival Study

Month April 1985 Location New Orleans

Midnight to Midnight

Date	Radiation		Temperature				Humidity				Rain(mm) Total
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		
1	88.9	1572.6	22	10	23.7	14.7	100	27	98.7	55	19
2	255.6		20	9			100	29			
3	250.0		24	6			100	48			
4	344.5		24	12			99	56			
5	22.2		23	18			98	78			
6	233.41		22	14			99	38			3
7	461.2		27	13			96	46			
8	522.4	1566.9	21	13	23	12.1	75	23	95.3	45.4	
9	544.6		21	10			97	36			
10	439.0		22	6.5			100	38			
11	461.2		21	12			98	64			
12	183.3		20	17			98	82			2
13	322.3		23	15.5			99	62			
14	311.2		25	13			100	58			10
15	430.1	2713.5	26	16	22.6	12.8	100	42	98.9	54.6	3 15
16	377.9		26	14			98	54			
17	394.5		27	12			99	45			
18	472.3		26	13			99	44			
19	33.3		25	14			99	70			18
20	394.5		26	15			100	67			
21	472.3		26	18			99	62			
22	333.4	2478.2	27	23	26.1	15.6	98	74	98.8	59.4	18
23	305.6		27	20			98	70			
24	300.1		28	20			100	65			
25	344.5		28.5	18			98	61			
26	327.8		28	18			99	63			
27	322.3		29	22			99	64			3
28	316.7		30	19			99	57			
29	372.3	2089.3	32	21	28.9	19.7	99	48	83.1	61.1	3
30	400.1	672.4	31	21	31.5	21	99	58			4
31											

Monthly Total 9857.5 Mean Hi 25.3 Mean Lo 15.9 Average 70

TABLE D-1 (Continued)

WEATHER STATION DATA

Ascaris Egg Survival Study

Month May 1985 Location New Orleans
Midnight to Midnight

Date	Radiation		Temperature				Humidity				Rain(mm)	
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily H	Daily L	Weekly Ave		Daily	Weekl Total
1	377.9		29	21			99	61				
2	516.8		28	18			98	55				
3	477.9		24	13			98	63				
4	544.6		26	18			98	53				
5	444.6		27	22			98	65				
6	266.76	2688.7	27	19	27.4	18.8	98	60	98.2	59.3		4
7	255.64		26	20			100	75			4	
8	372.35		28	18			100	60				
9	389.02		28	18			98	46				
10	377.91		30	18			99	52			7	
11	339.0		29	19			99	62			5	
12	355.68		30	20			99	59				
13	377.9	2467.5	29	21	28.6	19.1	100	61	99.3	59.3		16
14	527.9		30	21			100	60				
15	433.4		30	18			98	52				
16	477.9		31	14			100	29				
17	466.8		28	19			98	39				
18	450.1		25	15			97	50				
19	450.1		28	15			100	53				
20	77.3	2884.9	31	18	29	17.1	97	56	98.6	47		
21	194.5		28	20			100	69			9	
22	383.4		30	20			100	61			22	
23	94.4		25	18			99	80			8	
24	389.0		28	18			99	45				
25	439.0		29	14			99	36				
26			30	14			98	36				
27	322.3	1822.5 6days	29	16	28.4	17.1	99	41	99.1	52.6		39
28	411.2		29	18			99	47				
29	411.2		30	19			99	56				
30	389.0		31	21			100	53				
31	433.4	1967.1	32	23			99	60				

Monthly Total 11825.1 Mean Hi 28.5 Mean Lo 18.3 Average 74.04

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

June, 1985
Midnight to Midnight

New Orleans

Date	Radiation		Temperature				Humidity				
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		Rain (mm)
1	327.9		32	23			98	60			
2	389.0		33	21			100	56			
3	394.6	2756.3	32	21	31.3	20.9	98	51	99.0	54.7	
4	377.9		31.5	20			96	54			
5	466.8		33.5	19			98	44			5
6	472.4		33	21.5			99	50			
7	389.0		33	22			99	56			
8	322.3		32	24			99	65			
9	333.5		33	24			100	49			2
10	222.3	2194.5	30	24	32.3	22.1	99	63	98.6	54.4	
11	200.1		25	20			98	80			
12	411.3		29	20			97	56			6
13	439.0		27.5	19			97	50			25
14	283.4		28	19			100	73			5
15	194.5		27	24			100	85			
16	333.5		32	22			100	69			
17	322.3	2184.2	29	122	28.2	20.9	99	85	98.7	71.1	36
18	300.1		29	21			98	66			
19	289		29	21			98	53			
20	206		26	21			98	74			2
21	277.9		27	21			99	72			3
22	377.9		30	23			99	61			
23	411		31	19.5			100	52			
24	394.6	2256.5	30	21	28.9	21.1	99	46	98.7	60.6	5
25	344.6		31.5	20			97	46			
26	472.4		32	20			98	45			
27	355.7		32	21			98	57			
28	372.4		30	21			98	59			
29	344.6		27.5	20			98	57			
30	427.9		30	17			98	39			
31											

Radiation Total -10,064.3
Temperature Mean High - 30.2 Mean Low- 20.5
Humidity Average - 98.2 54.4
Rain (mm) Total - 69

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

July. 1985
Midnight to Midnight

New Orleans

Date	Radiation		Temperature				Humidity				
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		Rain (mm)
1	283	2600	32.5	19	30.6	19.7	100	51	98.1	50.6	5
2	385		31.5	20			100	55			
3	322		31	21			100	62			
4	245		29	21			100	68			3
5	100		26	21			100	91			13
6	150		26	21			100	90			16
7	161		25	22			99	91			7
8	250	1613	31	22	28.5	21.1	100	66	99.9	82	
9	384		31.5	23			100	64			
10	339		32	24			99	62			
11	361		33.5	22			98	55			
12	217		31	22.5			99	63			7
13	389		32.5	20.5			99	55			12
14	367		32.5	21.5			98	54			
15	150	2207	32	22	32.1	22.2	90	78	97.6	61.6	
16	72.2		28	24			91	72			15
17	411.3		26	21			91	60			
18	283.4		31	23			91	59			2
19	299		32	23			91	68			
20	311.2		31	23			91	62			3
21	311.2		30	23			91	55			
22	144.9	1833.2	33.5	--	30.2	22.8	92	58	91.5	62	
23	294.5		32	23			92	58			9
24	261.2		31	23.5			92	62			23
25	239		31	24			92	66			21
26	266.8		31	23			92	63			7
27	189		30	24			92	67			5
28	227.9		--	--			92	60			
29	155.6	1634	32	24	31.2	23.6	92	58	92	62	9
30	255.6		33	24			92	60			7
31	289		32	24.5			92	59			8

Radiation Total - 8,114.8
Temperature - Mean High 31.3 Mean Low 24.5
Humidity Average - 78.8
Rain (mm) Total - 179.0

TABLE D-1 (Continued)

WEATHER STATION DATA
***Ascaris* Egg Survival Study**

August, 1985
Midnight to Midnight

New Orleans

Date	Radiation		Temperature				Humidity				Rain (mm)
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		
1	250.0		33	23			92	72			31
2	322.3		33	24			92	70			
3	261.2		31	24			92	70			
4	361.2		32	24			92	60			2
5	183.4	1661.5	31	24	32.1	23.9	92	62	92	64.7	4
6	255.6		32	22			92	66			
7	150.0		31	22			92	62			7
8	361.2		32	24			92	58			18
9	350.1		34	24			92	48			17
10	389.0		34	24			92	50			8
11	394.6		34	24			91	52			
12	283.4	2183.9	32	23	32.7	23.2	94	55	92.1	55.8	
13	355.7		33	25			92	55			
14	*		*	*			*	*			3
15	*		*	25			97	72			21
16	305.7		31	25			96	70			44
17	350.1		33	24			95	54			8
18	250.0		29	24			94	51			
19	*	1261.5	*	*	31.5	24.6	*	*	94.8	60.4	
20	400.1		32.5	24			94	62			7
21	250.0		32	33			94	61			9
22	289		33	23			94	52			
23	350.1		33	24			94	49			
24	361.2		33	22			95	52			
25	227.9		32	22			95	75			
26	166.7	2045.0	30	21	32.6	23	94	62	94.3	58.5	
27	183.4		26	22			95	65			
28	344.6		30	25			97	63			
29	161.2		30	24			96	*			
30	*		*	*			*	*			
31	*		*	*			*	*			

*Hurricane warnings

**Radiation Total - 6701.8
**Temperature - Mean High 32.2   Mean Low 23.7
**Humidity Average - 76.6
**Rain (mm) Total - 170

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

September, 1985 **New Orleans, LA**
Midnight to Midnight

Date	Radiation		Temperature				Humidity					
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		Rain (mm)	
1	368.2		32	25			87	61				
2	4.0	1801.6	27	21	30.0	23.3	88	54	90.3	59.4	37	48
3	56.0		27	22			88	58			15	
4	304.2		32	24			85	61				
5	176.1		30	22			90	57				
6	364.1		31	22			90	54			26	
7	284.1		31	22			87	67				
8	316.1		32	22			90	56			1	
9	352.1	1852.7	31	21	30.6	22.1	88	54	83.6	58.1		42
10	216.0		31	21			85	50			31	
11	316.6		32	21			88	52				
12	372.3		33	22			87	61				
13	368.0		31	23			87	57				
14	119.8		27	22			88	69				
15	402.2		26	19			82	72				
16	380.8	2174.8	28	21	29.6	23.3	88	50	86.4	58.7		31
17	363.7		29	19			88	57				
18	359.4		29	19			88	52				
19	393.6		30	19			90	57				
20	372.2		30	21			88	56				
21	368.0		30	21			88	54				
22	248.2		30	22			87	57			5	
23	74.6	2179.7	28	23	29.4	20.6	85	72	87.7	57.9	3	8
24	155.6		29	21			87	74				
25	250.0		30	22			84	50				
26	177.8		25	18			85	52				
27	361.2		25	14			65	54				
28	227.8		27	13			73	52				
29	188.5		31	18			82	51			12	
30	78.3	1439.2	29	18	27.9	19.0	85	74	80.1	57.9	25	37
31												

Radiation Total - 9448.0
Temperature - Mean High 29.5 Mean Low 21.7
Humidity Average - 85.6 High 58.4
Rain (mm) Total - 155

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

October, 1985
Midnight to Midnight

New Orleans, LA

Date	Radiation		Temperature				Humidity				Rain (mm)
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		
1	77.8		20	15			94	70			2
2	216.7		21	14			94	71			
3	277.9		26	18			96	66			
4	261.2		30	18			94	63			
5	355.7		23	14			94	38			
6	427.9		23	9			93	33			
7	272.3	1889.5	25	13	24	14.4	94	50	94.3	55.9	2
8	239		25	22			94	65			3
9	355.7		26	19			93	67			3
10	316.8		29	19			94	53			
11	277.9		29.5	18			93	60			
12	227.9		30.5	19			93	60			
13	283.4		31	20			04	56			
14	194.5	1695.2	30	19.5	28.7	19.5	95	71	93.7	53.9	6
15	44.5		28	21			95	78			18
16	216.7		26	19			94	77			11
17	233.4		28.5	20			94	70			21
18	266.7		29	22			95	75			18
19	244.5		30	22			95	68			10
20	277.8		29	21			95	73			
21	200.1	1483.7	29	19.5	28.5	20.6	95	72	94.5	73.3	78
22	12.4		29	22			96	73			
23	344.6		29	21			95	65			
24	127.8		27.5	22			95	86			11
25	166.7		25	22			95	77			1
26	38.9		23.5	22			96	85			21
27	23.2		21	19			95	93			73
28	24.2	737.8	25.5	21.5	25.8	21.4	95	79	95.3	79.7	106
29	47.9		23	21			96	88			2
30	189.0		20.5	18			95	87			2
31	83.4		20	15			93	77			7

Radiation Total - 6006.2
Temperature - Mean High 26.8 Mean Low 19.0
Humidity Average - 94.5 High 65.7
Rain (mm) Total - 203

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

November, 1985 **New Orleans, LA**
Midnight to Midnight

Date	Radiation		Temperature		Humidity		
	Daily	Weekly Total	Daily H-L	Weekly Ave	Daily	Weekly Ave	Rain (mm)
1	273.3		23 14		93 62		4
2	111.2		18 15		92 51		
3	100.0		16 12		92 65		
4	222.3	1027.1	22 7	20.4 14.6	95 35		4
5	300.1		20 7		95 35		
6	244.6		27 16.		94 52		
7	272.3		24 12.5		93 46		
8	288.1		20 8		97 46		
9	270.1		25.5 10		97 52		
10	240.1		26 13		94 69		
11	114.0	1729.3	25 15	23.9 11.6	94 64	94.9 45.4	
12	233.4		26 18		95 73		
13	150.1		25 19		94 73		
14	189.0		26 20		94 67		
15	177.8		27 19		97 62		
16	205.6		28 20		98 62		
17	183.4		27 17		95 57		
18	261.2	1361.6	28 18	26.7 18.7	95 66	95.4 65.7	
19	261.2		28 18		95 57		
20	266.8		24 19		94 60		
21	222.3		22 16		94 64		
22	205.6		22 14		94 58		
23	189.0		24 12		94 56		
24	155.6		24 14		95 69		
25	133.4	1433.9	26 18	24.3 15.9	94 62	94.3 61	
26	205.6		28 22		94 73		
27	144.5		26 21		94 79		14
28	155.6		22 18		94 79		6
29	55.6		20 19		94 93		1
30	55.6		21.5 18		94 86		
31							

Radiation Total - 5626.2
Temperature - Mean High 23.8 Mean Low 15.2
Humidity Average - 95.7 High 63.6
Rain (mm) Total - 25

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

December, 1985
Midnight to Midnight

New Orleans, LA

Date	Radiation		Temperature				Humidity				Rain* (mm)
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		
1	166.7		12	6			94	64			
2	294.5	1978.1	14	9	20.5	16.1	77	33	91.6	72.4	
3	316.8		13	3			85	37			
4	277.9		19	3			96	53			
5	216.7		17	8			94	63			
6	255.6		17	4			94	35			
7	272.3		18	3			95	60			
8	194.5		21	8			95	52			
9	244.5	1778.3	22	15	18.1	6.3	94	58	94.7	51.1	
10	183.4		24	15			94	65			
11	122.3		25	18			94	75			
12	105.6		21	15			95	87			
13	77.8		17	6			95	64			
14	344.6		7	-1			86	30			
15	383.5		9	-3			93	33			
16	327.6	1544.8	17	0	17.1	7.1	93	22	92.3	53.7	
17	296.5		16	-1			95	26			
18	326.8		12	4			94	47			
19	344.6		12	0			95	40			
20	267.4		13	-1			95	27			
21	278.5		13	0			95	30			
22	361.2		18	-1			95	30			
23	339.0	2214.0	22	9	15.14	1.4	95	56	94.8	36.6	
24	239.0		18	8			93	31			
25	300.1		4	1			57	32			
26	300.1		9	-6			93	25			
27	55.6		18	5			94	56			
28	77.8		14	10			94	88			
29	216.7		17	6			94	35			
30	166.7	1356.0	20	1	14.3	3.6	94	38	88.4	43.6	
31											

Radiation Total - 735.3
Temperature - Mean High 17.0 Mean Low 4.9
Humidity Average - 92.4 High 51.5

* Rain gauge mechanism in repair shop since 12/13/85 - no rain for this month up to this date. Will finish complete rainfall data in next quarterly report.

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

Month January, 1986 New Orleans, LA
Midnight to Midnight

Date	Radiation		Temperature		Humidity		
	Daily	Weekly Total	Daily H-L	Weekly Ave	Daily	Weekly Ave	Rain (mm)
December							
30	166.13		20 11.5		94 38		
31	111.15		22 13		95 79		
1	194.5		15 5		94 46		
2	183.4		20.5 6		95 57		
3	194.5		20 10		94 40		
4	27.8		15 10		95 50		
5	261.2		12 0		92 27		
6	155.6	1128.2	10 -2	16.4 6.3	93 56	94 50.7	
7	50.0		11 8		93 49		
8	266.8		13 6		54 31		
9	161.2		8.5 6		94 48		
10	77.8		8.8 6.5		94 63		
11	266.8		15 2		94 33		
12	277.9		18 0		95.5 26		
13	227.9	1328.4	19 1	13.3 4.2	95 14	88.5 37.7	
14	222.3		19.5 -1		87.5 18.5		
15	194.5		21 2		88.5 26		
16	211.2		17 2		88.5 61		
17	205.6		20 10		88 62.5		
18	189.0		20 11		88 66		
19	244.5		20 6		88 19		
20	227.9	1495.0	27 3	20.6 4.7	88 29	88.1 40.3	
21	138.9		24 7.5		88 40		
22	105.6		24 10.5		88 44		
23	127.8		15 5		87 34		
24	111.2		18.5 1.5		91 36		
25	83.4		20 8		89 57		
26	172.3		13 7. 5		87 26.5		
27	183.4	922.6	6 -1	17.2 5.6	72 18	86 36.5	
28	161.2		16 -4		88 35		
29	144.5		22 4		89 45		
30	116.7		17.5 8		88 39		
31	177.8		21.5 6		90 50		

Radiation Total - __5363.2__
Temperature - Mean High __17.0__ Mean Low __4.7__
Humidity Average - __88.9__ - __40.2__
Rain (mm) Total -

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

Month February, 1986 New Orleans, LA
Midnight to Midnight

Date	Radiation		Temperature		Humidity		
	Daily	Weekly Total	Daily H-L	Weekly Ave	Daily	Weekly Ave	Rain (mm)
1	166.7		23 12		90 54		
2	138.9		24 11		90 48		
3	38.9	944.7	26 13	21.4 7.1	89 45	89.1 45.1	
4	72.2		25 15		89.5 63		
5	133.4		23 16.5		89.5 65		
6	250.1		22 13		89 27		
7	272.3		20 9		89 68		
8	172.3		20.5 14		89 55		
9	150.1		17 13.5		89.5 66		
10	25.0	1075.4	16.5 2	20.6 11.9	90 65	89.4 58.4	
11	32.5		4 -2		75 55		
12	187.6		8.5 -2		79 27		
13	225.1		12 1		87 38		
14	25.0		22 5		89 55		
15	230.1		17 4.5		91 25		
16	75.0		24 12		88.5 54		
17	289.0	1064.3	24.4 17.5	16.0 5.1	91 63	85.8 45.3	
18	250.1		25.5 17		91 56		
19	200.1		25.5 16.5		90 53		
20	211.2		25 15		90 56		
21	150.1		25 15.5		90 53		
22	111.2		17.5 10		90 61		
23	227.9		19 6		90 22		
24	66.7	1217.3	22.5 5	22.9 12.1	91 28	90.3 48	
25	283.4		20.5 6		89 25		
26	277.9		25 9		90 44		
27	227.9		25 11		86.5 24		
28	227.9		16 2.5		80 25		
29							
30							
31							

Radiation Total - __4718.6__
ture - Mean High __20.6__ Mean Low __9.6__
Humidity Average - __88.3__ - __47.4__
Rain (mm) Total -

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

Month March, 1986 New Orleans, LA
Midnight to Midnight

Date	Radiation		Temperature		Humidity		
	Daily	Weekly Total	Daily H-L	Weekly Ave	Daily	Weekly Ave	Rain (mm)
1	311.2		*		*		
2	283.4		20 1		92.5 37		
3	50.0	1661.7	23 10		94 42		
4	211.2		21 11		93 26		
5	244.5		20 3		93 35		
6	289.0		22 7.5		95 18		
7	300.1		24 3		95 20		
8	261.2		22 5		96 47		
9	200.1		24 10		96 62		
10	*		27.5 15		95 76		
11	*		25.5 19		95 67		
12	*		27 15		98 66		
13	*		26.5 13		96 39		
14	*		27 13		95 35		
15	*		28 12		92 39		
16	*		30 11		90 26		
17	*						
18	*						
19	*						
20	*						
21	*						
22	*						
23	*						
24	255.5						
25	227.9						
26	222.3						
27	261.2						
28	305.7						
29	305.7						
30	266.8						
31	322.3						

Radiation Total -
Temperature - Mean High ______ Mean Low ______
Humidity Average -
Rain (mm) Total -

* Not available - data will be compiled for next quarterly report.

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

Month April, 1986
Midnight to Midnight

New Orleans, LA

Date	Radiation		Temperature		Humidity		Rain
	Daily	Weekly Total	Daily H-L	Weekly Ave	Daily	Weekly Ave	(mm)
1	300.1						
2	250.1						
3	216.7						
4	189.0						
5	222.3						
6	283.4		Data		Data		
7	211.2	1672.8					
8	222.3		not		not		
9	283.4						
10	72.2		available		avail-		1
11	294.5				able		1
12	161.2		because				3
13	227.9						3
14	225.6	1487.1	weather				1
15	272.3						1
16	350.1		station				
17	361.2						
18	366.8		blew				
19	200.1						8
20	205.6		over				30
21	194.5	1950.6					30
22	222.3						
23	222.3						
24	222.4						
25	244.5		27 14.5		100 50		
26	222.3		27 12		99 52		
27	194.5		28 15		98 55		
28	177.8	1506.0	28.5 19	27.6 15.1	97 49		
29	189.0		29 15.5		98 40		
30	244.5		29 16.5		97 47		
31							
31	177.8		21.5 6		90 50		

Monthly Total 7050
Temperature - Mean High 28.1 Mean Low 15.4
Humidity Average - 98.2 - 48.8
Rain (mm) Total - 78

TABLE D-1 (Continued)

WEATHER STATION DATA
Ascaris Egg Survival Study

Month May, 1986
Midnight to Midnight

New Orleans, LA

Date	Radiation		Temperature				Humidity				
	Daily	Weekly Total	Daily H-L		Weekly Ave		Daily		Weekly Ave		Rain (mm)
1	311.2		31	20			96	46			
2	233.4		28	19			96.5	40			
3	333.5		28	16.5			93	26.5			
4	361.2		29.5	12.5			97	33			
5	138.9	1811.7	28	18.5	28.9	16.9	96	52	96.2	40.6	
6	172.3		30	19			99	54			
7	250.1		30	23			97	54			
8	255.6		30.5	20			97	55			
9	283.4		33	20.5			97	37.5			
10	144.5		31	20			96.5	59			
11	*	1105.9	29	23.5			96	60			
12	222.3		31	25	30.6	21.6	90	32	96.1	45.6	
13	189.0		31	22			90	46			
14	222.3		32	20.5			89	46			
15	172.3		28	23			88	62			
16	250.1		28.5	21.5			99	54			
17	100.0		27	23			99	70			
18	222.3	1378.3	29	21	29.3	21.8	100	57	94.2	55.8	
19											
20											
21											
22											
23											
24											
25											
26											
27											
28											
29											
30											
31											

Radiation Total - __4295.9__
Temperature - Mean High __29.6__ Mean Low _20.5__
Humidity Average - __95.3__ - __49.1__
Rain (mm) Total - 23

APPENDIX E

DIGESTER OPERATIONAL DATA

TABLE E-1. PERCENT DESTRUCTION OF VOLATILE SOLIDS BY DIGESTERS FOR FIRST RUN

DATE	DIGESTER			
	1	2	3	4
8/2	22.5	50.4	117.8/600[a]	60.5
8/5	99.3	81.0	63.9	68.7/700
8/8	2.6	15.5	27.5	9.0
8/12	39.6	50.0	(74.8)	37.9/1400
8/15	59.6	26.2	86.8	76.6
8/17	125	107	84.8	111.7
8/22	(-30.4)	20.6	(-34)	(-22.2)
8/26	(-39.6)	(-59.9)	(-6.3)	37.5/670
8/29	24.7/1770	55	70.0	50.2
9/2	122/403	97	61.4	55.0
9/6	55.3/766	(-4.9)	54.6	61.3
9/9	(-11.3)	44.4	(-17.4)	11.9
9/12	39.6	67.6/914	114.5	91.8
9/16	78.2	53.9/1270	34.5	42.7
9/19	(-2.1)	59.1/937	58.7	62.2
9/23	175.8	158.7	150.3	134.9
9/26	0.4	(-36.1)	(-65.7)	(-15.7)
9/30	53.7	48.3/1269	88.5	58.0
MEAN	45.3/980	46.3/1098	45.3/-	51.8/923

[a] Liters of gas produced per kg volatile solids destroyed

TABLE E-2. DIGESTER OPERATIONAL DATA FOR FIRST RUN

DATE	DIGESTER NO.	PH	TEMP ^{o}C	GAS PRODUCED L	GAS PRODUCED L/Rg VS DESTROYED	TOTAL SOLIDS G/L	VOLATILE SOLIDS G/L	%VS DESTROYED	ALKALINITY MG/L $CaCO_3$
10/3	1	7.1	35	+	-	33.0	14.2	37.1	4075
	2	7.1	35	21.4	1392	37.0	14.4	36.5	7075
	3	7.0	35	+	-	32.8	14.0	35.6	4075
	4	7.0	35	+	-	21.9	12.8	57.9	4025
10/7	1	7.1	35	+	-	32.8	13.8	70.0	4050
	2	7.1	35	30.5	918	32.6	13.7	74.5	3800
	3	7.0	35	+	-	29.9	13.5	71.2	4000
	4	7.1	35	+	-	25.9	13.3	51.2	4000
10/11	1	7.2	35	+	-	41.5	12.8	77.8	4000
	2	7.2	35	21.5	1913	38.5	14.2	34.0	4175
	3	7.1	35	+	-	28.7	13.0	64.2	4000
	4	7.1	35	+	-	22.7	12.0	88.9	4100
10/14	1	7.0	35	+	-	36.1	13.9	14.5	4400
	2	7.0	35	16.2	1685	35.0	14.6	30.6	4050
	3	7.0	35	+	-	35.5	12.6	59.3	3775
	4	7.0	35	+	-	23.7	12.4	42.0	3900
10/18	1	6.9	35	+	-	38.1	14.3	41.0	4175
	2	7.0	35	+	-	37.4	13.7	73.3	4100
	3	7.0	35	24.0	1150	34.2	12.5	58.4	4175
	4	7.0	35	+	-	21.6	12.3	59.1	3850

(Continued)

TABLE E-2 (continued)

DATE	DIGESTER NO.	PH	TEMP ^{o}C	GAS PRODUCED L	GAS PRODUCED L/Rg VS DESTROYED	TOTAL SOLIDS G/L	VOLATILE SOLIDS G/L	%VS DESTROYED	ALKALINITY MG/L $CaCO_3$
10/21	1	6.8	35	+	-	33.2	13.7	66.7	3700
	2	6.8	35	+	-	33.7	14.1	41.0	4300
	3	6.8	35	24.6		32.5	14.4	-	3700
	4	6.8	35	+	-	31.7	14.2	-	3950
10/24	1	6.9	35	+	-	33.2	14.2	42.2	3775
	2	7.0	35	+	-	33.7	15.0	29.3	3650
	3	7.0	35	23.4	-	32.5	17.1	-	3950
	4	6.9	35	+	-	31.7	19.0	-	4400
10/28	3	6.8	35	24.0	2808	27.1	16.5	35.6	-
	4	6.8	35	+	-	31.5	18.1	38.2	-
10/31	3	7.0	35	10.6	638	32.5	16.1	50.0	5000
	4	7.0	35	+	-	32.2	17.1	62.0	5700
11/4	3	7.0	35	20.4	971	37.2	14.9	72.1	4775
	4	7.0	35	+	-	33.0	15.9	67.8	5475
11/7	3	7.0	35	16.0	847	33.3	13.9	68.6	4450
	4	7.0	35	+	-	31.2	14.9	64.1	5500
11/11	3	7.1	35	22.4	1030	36.8	13.5	61.9	4250
	4	7.1	35	+	-	32.7	14.9	47.0	4975
11/14	3	7.0	35	16.1	1533	24.6	13.8	34.6	4250
	4	7.0	35	+	-	26.3	14.1	65.0	4975

(Continued)

TABLE E-2 (continued)

DATE	DIGESTER NO.	PH	TEMP ^{o}C	GAS PRODUCED		TOTAL SOLIDS G/L	VOLATILE SOLIDS G/L	%VS DESTROYED	ALKALINITY MG/L $CaCO_3$
				L	L/Rg VS DESTROYED				
11/21	3	7.1	35	16.1	-	34.8	16.7	-	4800
	4	7.1	35	+	-	31.9	16.7	-	3825
11/26	3	7.0	35	35.2	1429	28.7	15.9	65.6	-
	4	7.1	35	+	-	26.5	15.4	81.7	-
11/29	3	6.9	35	22.0	3257	32.3	17.4	16.2	4050
	4	7.0	35	+	-	28.6	14.3	78.9	4500
12/2	3	6.9	35	49.6	-	44.9	23.4	-	5100
	4	6.8	35	+	-	42.7	22.3	-	5050
12/5	3	6.7	35	63.5	-	55.5	29.8	-	5750
	4	6.7	34	+	-	49.4	29.2	-	4475
12/9	3	7.0	34	40	2644	50.1	28.3	40.5	5225
	4	6.9	34	+	-	49.7	29.2	55.9	4525
12/12	3	6.9	35	+	-	43.9	24.5	106.5	5100
	4	6.8	35	+	-	43.1	23.8	100.0	4600
12/16	3	7.0	35	30.8	816	43.2	21.4	101.5	5100
	4	7.0	35	+	-	43.7	21.6	79.3	4650
12/17	3	7.0	35						
	4	6.9	35						

APPENDIX F

BACTERIAL DATA

TABLE F-1. *SALMONELLA* QUALITY ASSURANCE DATA

A) New Orleans lagoon spike concentrations:[a]

	Spike	/	Recovery
Aqueous fraction:	4.0×10^8	/	107%
Sediment fraction:	9.3×10^7	/	99%

B) Port Aransas lagoon spike concentrations:[a]

	Spike	/	Recovery
Aqueous fraction:	1.5×10^8	/	102%
Sediment fraction:[b]	1.4×10^6	/	76.1%

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[a] Cultures used for quality assurance were at a concentration of 1.1×10^8 (MPN enumeration)

[b] Possible toxic effect of Port Aransas sludge fraction on the *Salmonella*

TABLE F-2. NEW ORLEANS LAGOON BACTERIAL COUNTS PER 100 ML

February 6, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
1-1	1.1×10^{6}	1.2×10^{5}	4.3×10^{4}
2-1	1.0×10^{6}	1.3×10^{5}	4.0×10^{4}
3-1	1.4×10^{6}	1.0×10^{5}	2.3×10^{4}
4-1	1.0×10^{6}	1.3×10^{5}	2.0×10^{4}
5-1	1.0×10^{6}	1.1×10^{5}	2.7×10^{4}
5-1Q	1.0×10^{6}	1.3×10^{5}	2.5×10^{4}
1-2	6.7×10^{5}	8.0×10^{4}	1.3×10^{4}
2-2	1.5×10^{6}	1.4×10^{5}	4.0×10^{4}
3-2	1.3×10^{6}	1.1×10^{5}	1.0×10^{4}
4-2	1.6×10^{6}	1.3×10^{5}	5.0×10^{4}
5-2	1.9×10^{6}	1.2×10^{5}	3.0×10^{4}
5-2Q	1.8×10^{6}	1.0×10^{5}	1.7×10^{4}

March 12, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
1-1	3.3×10^{4}	8.1×10^{3}	2.3×10^{2}
2-1	2.8×10^{4}	6.2×10^{3}	3.3×10^{1}
3-1	3.7×10^{4}	5.5×10^{3}	2.7×10^{2}
4-1	4.0×10^{4}	6.5×10^{3}	3.0×10^{2}
5-1	3.9×10^{4}	5.7×10^{3}	3.0×10^{2}
5-1Q	2.9×10^{4}	6.0×10^{3}	2.0×10^{2}
1-2	2.0×10^{5}	2.0×10^{4}	5.7×10^{3}
2-2	1.4×10^{5}	1.9×10^{4}	8.7×10^{3}
3-2	1.9×10^{5}	1.9×10^{4}	8.0×10^{3}
4-2	1.4×10^{5}	1.5×10^{4}	9.0×10^{3}
5-2	2.5×10^{5}	6.1×10^{4}	1.8×10^{4}
5-2Q	2.2×10^{5}	5.9×10^{4}	1.5×10^{4}

(Continued)

TABLE F-2 (continued)

April 9, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
1-1	1.6×10^4	2.6×10^2	2.3×10^2
2-1	1.2×10^4	5.0×10^2	2.3×10^2
3-1	1.2×10^4	2.3×10^2	2.4×10^2
4-1	1.1×10^4	-	1.6×10^2
5-1	6.5×10^3	2.0×10^1	2.4×10^2
5-1Q	6.2×10^3	2.3×10^1	2.0×10^2
1-2	2.4×10^5	1×10^3	2.0×10^3
2-2	4.0×10^5	1×10^3	2.0×10^3
3-2	2.0×10^5	1×10^3	1.5×10^3
4-2	8.0×10^4	-	2.0×10^3
5-2	9.5×10^4	2×10^3	2.0×10^3
5-2Q	4.2×10^4	2×10^3	2.0×10^3

May 7, 1984

Coliforms assays results are only for the aqueous portion of samples due to high solids content of sludge which clogged the filters.

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
1-1	8.0×10^2	1.3×10^1	1.8×10^1
2-1	5.7×10^2	2.7×10^1	1.4×10^1
3-1	3.7×10^2	1.7×10^1	1.5×10^1
4-1	3.0×10^2	4.7×10^1	1.5×10^1
5-1	1.7×10^2	4.7×10^1	1.7×10^1
5-1Q	2.0×10^2	4.7×10^1	1.6×10^1

(Continued)

TABLE F-2 (continued)

June 18, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORMS	TOTAL STREPTOCOCCI
1-1	4.3×10^4	None detected up to solids limitations	2.0×10^3
2-1	1.0×10^4		4.0×10^3
3-1	8.3×10^4		1.1×10^4
4-1	2.3×10^4		5.0×10^3
5-1	1.0×10^4		2.3×10^3
5-1Q	2.0×10^4		3.0×10^3
1-2	None detected up to solids limitations	None detected up to solids limitations	None detected up to solids limitations
2-2			
3-2			
4-2			
5-2			
5-2Q			

(Continued)

NOTES:

These sampling results showed that the membrane filter technique could not be used any further on this project. The presence of algae in surface waters prevented larger amounts of aqueous portions to be filtered, while bottom sediment samples contained too much solids for reliable results.

The odor of ammonia was noted above the lagoon's surface waters prior to sampling. Green photosynthetic single cellular flagellates were growing well on the top layers of the aqueous portion. When bottom samples were collected with a coliwasa-type sampler, this yielded better recovery from sediment sands.

(Continued)

TABLE F-2 (continued)

August 6, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
3-1			
5-1	2.4×10^4	4	4.6×10^4
5-1Q	4.3×10^3	43	2.4×10^4
5-1Q	4.3×10^3	460	2.4×10^4
3-2	2.4×10^{10}	<3	4.6×10^4
5-2	1.5×10^8	<3	2.4×10^4
5-2Q	1.2×10^8	4	2.4×10^4

NOTES:

There were problems with fecal coliform enumeration as numbers were not consistent. Algae was plentiful and there was plenty of mosquito larvae and other insects - spiders, dragonflies, etc.

November 14, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
3-1	2.4×10^3	None detected	6×10^2
5-1	1.1×10^3	None detected	1.1×10^3
5-1Q	1.1×10^3	None detected	4.6×10^2
3-2	2.4×10^4	None detected	4.6×10^3
5-2	1.1×10^4	None detected	1.1×10^4
5-2Q	1.1×10^4	None detected	1.1×10^4

May 14, 1985

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORMS	TOTAL STREPTOCOCCI
3-A	8.7×10^3	<2.0	5.0
3-S	1.6×10^4	2.67	2.6×10^1

(Continued)

TABLE F-2 (continued)

December 19, 1985			
3-A	1.6×10^4	<3	4.8×10^3
3-S	3.1×10^2	<3	2.0×10^3
May 1, 1986			
3-A	1.7×10^3	3.63	3.2×10^3
3-S	1.1×10^4	<3	7.3×10^3

NOTES:
Lagoon condition: water level was about six feet from top of box. Fungi and lichen were growing on the interior near the water level. Top samples were highly colored with green algae and had large numbers of mosquito larvae. Sediment samples were almost odorless, but still high in solids content although finer grained.

Samples were homogenized in a blender before coliform and Salmonella analysis. This seemed to assist in better distribution of organisms in the multi-tube assay.

TABLE F-3. PORT ARANSAS LAGOON BACTERIAL COUNTS PER 100 ML

February 24, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
1-1	3.0×10^8	1.2×10^7	2.9×10^6
2-1	3.3×10^8	8.3×10^6	3.2×10^6
3-1	4.0×10^8	8.9×10^6	2.6×10^6
4-1	2.6×10^8	9.0×10^6	2.3×10^6
5-1	4.9×10^8	9.7×10^6	1.9×10^6
5-1Q	4.7×10^8	1.2×10^7	1.6×10^6
1-2	3.4×10^8	8.3×10^6	3.4×10^6
2-2	4.0×10^8	8.6×10^6	2.6×10^6
3-2	2.9×10^8	7.5×10^6	2.5×10^6
4-2	2.5×10^8	7.0×10^6	2.2×10^6
5-2	1.6×10^8	7.3×10^6	1.6×10^6
5-2Q	1.7×10^8	6.5×10^6	2.5×10^6

April 4, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
1-1	5.3×10^5	1.3×10^5	6.7×10^3
2-1	4.3×10^5	1.8×10^5	1.1×10^4
3-1	1.1×10^6	1.9×10^5	2.9×10^4
4-1	1.1×10^6	2.1×10^5	2.0×10^4
5-1	5.7×10^5	1.3×10^5	7.0×10^3
5-1Q	6.7×10^5	1.7×10^5	1.1×10^4
	(10^{-4})	(10^{-2})	(10^{-2})
1-2	1.0×10^6	1.5×10^6	7.7×10^4
2-2	2.3×10^6	2.5×10^6	1.5×10^5
3-2	4.7×10^6	5.1×10^6	2.7×10^5
4-2	4.7×10^6	3.4×10^6	4.5×10^5
5-2	2.3×10^6	2.1×10^6	2.8×10^5
5-2Q	4.3×10^6	2.2×10^6	2.6×10^5

(Continued)

NOTE:

Solids began interfering in coliform determinations.

TABLE F-3 (continued)

May 7, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
1-1	1.1×10^5	1×10^2	2.4×10^4
2-1	1.1×10^5	0 none	1.8×10^4
3-1	3.6×10^4	0 present	1.2×10^4
4-1	5.7×10^4	0 at this	1.2×10^4
5-1	6.1×10^4	0 concentration	2.1×10^4
5-1Q	8.3×10^4	0	2.5×10^4
1-2	4.3×10^5	0 none	9.6×10^4
2-2	2.0×10^5	0 present	1.1×10^5
3-2	1.2×10^5	0 at this	1.1×10^5
4-2	2.6×10^5	0 concentration	2.4×10^5
5-2	1.1×10^5	1×10^2	1.1×10^5
5-2Q	1.7×10^5	0	9.9×10^4

June 4, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
1-1	7.5×10^4	0	4.3×10^2
2-1	1.9×10^5	0	3.1×10^2
3-1	3.0×10^4	0	2.2×10^2
4-1	9.5×10^4	0	3.1×10^2
5-1	9.0×10^4	10/100 mL	2.3×10^2
5-1Q	1.2×10^5		2.1×10^2
1-2	4.5×10^5	2.0×10^2	1.0×10^5
2-2	2.0×10^5	0	1.0×10^5
3-2	1.0×10^5	0	0
4-2	1.6×10^6	0	0
5-2	1.5×10^5	0	0
5-2Q	1.0×10^5	1×10^3	1.0×10^5

(Continued)

NOTE:

Solids were causing a real problem with enumeration of fecal coliform bacteria.

TABLE F-3 (continued)

July 2, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
3-1	1.1×10^5	<3	40
5-1	1.5×10^5	<3	230
5-1Q	9.0×10^4	<3	230
3-2	1.1×10^{10}	2.4×10^5	230
5-2	4.6×10^7	9.3×10^4	230
5-2Q	3.9×10^7	2.4×10^5	90

NOTES:
This is the first data set using the multiple tube fermentation technique for coliform determination. The filtration method was discontinued due to the high solids content of the sediment samples and declining coliform populations. Please note the higher bacterial counts in the sediment samples. This can only be attributed to the binding of organisms to the particulate matter present in these samples.

There was a greater difference in bacterial counts between top and bottom samples. The top samples were truly aqueous - with little solids present in comparison to the sediment samples. The coliform population decline followed this decrease in solids on which to feed in total and fecal coliforms. The fecal streptococci seemed to be more evenly dispersed through the phases than the other coliform organisms.

September 10, 1984

SAMPLE #	TOTAL COLIFORM	FECAL COLIFORM	FECAL STREPTOCOCCI
3-1	2.4×10^5	<3	240
5-1	9.3×10^4	<3	93
5-1Q	2.4×10^5	<3	240
3-2	2.8×10^6	240	4.3×10^3
5-2	4.3×10^6	240	2.4×10^4
5-2Q	2.3×10^6	240	1.5×10^3

NOTES:

Samples from aqueous portion of lagoon (3-1 & 5-1) were clear with green tinge due to algae. Sediment layer on bottom was 2 1/2 - 3 feet thick. Sediment was still very high in organic refractory waste and grease.

TABLE F-4. NEW ORLEANS TOTAL AND FECAL COLIFORM CONCENTRATIONS BY VOLUME

Date		Total Coliform /100 mL		Fecal Coliform /100 mL	
		Geometric Mean	Log G.M. Log S.D.	Geometric Mean	Log G.M. Log S.D.
02/06/84	A[a]	$1.07x10^{6}$ [c]	6.03±0.06	$1.19x10^{5}$	5.08±0.12
	S[b]	$1.39x10^{6}$ [c]	6.14±0.17	$1.12x10^{5}$	5.05±0.09
03/12/84	A	$3.40x10^{4}$	4.53±0.07	$6.28x10^{3}$	3.80±0.06
	S	$1.86x10^{5}$	5.27±0.10	$2.71x10^{4}$	4.43±0.27
04/09/84	A	$1.00x10^{4}$	4.00±0.16	$1.07x10^{2}$	2.03±0.65
	S	$1.35x10^{5}$	5.13±0.36	$1.32x10^{3}$	3.12±0.11
05/07/84	A	$3.46x10^{2}$	2.54±0.26	$2.92x10^{1}$	1.46±0.25
	S	$<9.13x10^{2}$ [d]	<2.96±0.00[d]	$<2.6\ x10^{3}$ [d]	<3.41±0.00[d]
06/18/84	A	$2.34x10^{4}$	4.37±0.36	$<3.8\ x10^{3}$ [d]	<3.58±0.00[d]
	S	$<9.66x10^{2}$ [d]	<2.98±0.00[d]	$<1.0\ x10^{3}$ [d]	<3.01±0.00[d]
08/06/84	A	$7.6\ x10^{3}$	3.88±0.43	$4.31x10^{1}$	1.63±1.03
	S	$7.6\ x10^{8}$	8.88±1.30	3.3	0.52±0.07
11/14/84	A	$1.43x10^{3}$	3.15±0.20	2.0	0.30±0.00
	S	$1.43x10^{4}$	4.15±0.20	2.0	0.30±0.00
05/14/85	A	$8.7x10^{3}$	3.90±0.24	<2.0	<0.30±0.00
	S	$1.6x10^{4}$	3.98±0.64	2.67	0.42±0.10
12/19/85	A	$1.6x10^{4}$	4.22±0.19	<3	<0.30±0.00
	S	$3.1x10^{2}$	2.50±0.16	<3	<0.30±0.00
03/01/86	A	$1.7x10^{3}$	3.22±0.69	3.63	0.56±0.07
	S	$1.1x10^{4}$	4.03±0.29	<3	0.48±0.00

(Continued)

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[a] Aqueous Sample [b] Sediment Sample

[c] Initial spike concentration/100 mL sludge theoretical

[d] Bacterial counts dropped to a level which became undetectable utilizing membrane filtration technique due to high solids concentration in some areas and all sediment samples. Assay was changed to multiple-tube fermentation technique 8/6/84.

TABLE F-4 (continued)

NEW ORLEANS FECAL STREPTOCCI AND SALMONELLA CONCENTRATIONS BY VOLUME

		Fecal Streptococci /100 mL			Salmonella /100 mL		
		Geometric Mean	Log G.M.	Log S.D.	Geometric Mean	Log G.M.	Log S.D.
02/06/84	A[a]	2.26x10^{4} [c]	4.35	±0.21	1.45x10^{7}	7.16	
	S[b]	2.26x10^{4} [c]	4.35	±0.28	1.45x10^{7}	7.16	
03/12/84	A	2.68x10^{2}	2.43	±0.08	8.23x10^{1}	1.92	±0.29
	S	9.91x10^{3}	3.99	±0.18	4.81x10^{2}	2.69	±0.58
04/09/84	A	2.15x10^{2}	2.33	±0.07	7.0 x10^{1}	1.85	
	S	1.86x10^{3}	3.27	±0.05	<3.0[d]	0.48	±0.00
05/07/84	A	1.59x10^{1}	1.20	±0.04	1.0	0.00	±0.00
	S	<1.7 x10^{3} [d]	<3.23	±0.00[d]	1.0	0.00	±0.00
06/18/84	A	3.79x10^{3}	3.58	±0.27	4.0 x10^{-1}	-0.40	±0.49
	S	<1.0 x10^{3} [d]	<3.01	±0.00[d]	7.0 x10^{-1}	0.15	±0.00
08/06/84	A	2.98x10^{4}	4.47	±0.15	4.19	0.62	±0.17
	S	2.98x10^{4}	4.47	±0.15	3.02	0.48	±0.28
11/14/84	A	6.22x10^{2}	2.79	±0.22	9.0x10^{-1}	-0.05	±0.00
	S	8.24x10^{3}	3.92	±0.0	2.3	0.36	±0.00
05/14/85	A	5.0	0.68	±0.14	2.6x10^{1}	1.42	±0.87
	S	2.6x10^{1}	1.43	±0.51	5.5x10^{-1}	-0.26	±0.36
12/19/85	A	4.8x10^{3}	3.68	±0.48	<3.0x10^{-1}	0.48	±0.00
	S	2.0x10^{3}	3.31	±0.09	<3.0x10^{-1}	0.48	±0.00
03/01/86	A	3.2x10^{3}	3.51	±0.15	<3.0x10^{-1}	0.48	±0.00
	S	7.3x10^{3}	3.86	±0.36	<3.0x10^{-1}	0.48	±0.00

[a] Aqueous Sample [b] SSediment Sample

[c] Initial spike concentration/100 mL sludge theoretical

[d] Bacterial counts dropped to a level which became undetectable utilizing membrane filtration technique due to high solids concentration in some areas and all sediment samples. Assay was changed to multiple-tube fermentation technique 8/6/84.

TABLE F-5. PORT ARANSAS BACTERIAL[c] CONCENTRATIONS BY VOLUME

		Total Coliform /100 mL			Fecal Coliform /100 mL			Fecal Streptococci /100 mL		
		Geometric Mean	Log G.M.	Log S.D.	Geometric Mean	Log G.M.	Log S.D.	Geometric Mean	Log G.M.	Log S.D.
02/25/84	A[a]	$3.65x10^{8}$	8.5625	±0.0103	$9.88x10^{6}$	6.9946	±0.0689	$2.35x10^{6}$	6.3712	±0.1138
	S[b]	$2.54x10^{8}$	8.4047	±0.1606	$7.50x10^{6}$	6.8750	±0.0455	$2.41x10^{6}$	6.3815	±0.1069
04/04/84	A	$6.87x10^{5}$	5.8371	±0.1699	$1.66x10^{5}$	5.2191	±0.0689	$1.17x10^{4}$	4.0682	±0.2520
	S	$2.74x10^{6}$	6.4385	±0.2583	$2.59x10^{6}$	6.4100	±0.1846	$2.16x10^{5}$	5.3349	±0.2668
05/02/84	A	$5.80x10^{4}$	4.7627	±0.2024	2.15	0.3333	±0.8165	$4.26x10^{3}$	3.6295	±1.5867
	S	$1.92x10^{5}$	5.2834	±0.2206	2.15	0.3333	±0.8165	$1.20x10^{5}$	5.0804	±0.1492
06/06/84	A	$8.72x10^{4}$	4.9403	±0.2662	1.58	0.2000	±0.4472	$2.76x10^{2}$	2.4404	±0.1202
	S	$2.62x10^{5}$	5.4184	±0.4658	7.65	0.8835	±1.3865	$1.00x10^{5}$	5.0000	±0.00
07/02/84	A	$1.14x10^{5}$	5.0572	±0.1118	5.01	0.7720	±0.5107	$1.28x10^{2}$	2.1085	±0.4386
	S	$2.70x10^{8}$	8.4317	±1.3945	$1.75x10^{5}$	5.2430	±0.2377	$1.68x10^{2}$	2.2259	±0.2353
09/10/84	A	$1.75x10^{5}$	5.2430	±0.2377	3.0	0.4771	±0.0008	$1.75x10^{2}$	2.2430	±0.2377
	S	$3.03x10^{6}$	6.4808	±0.1390	$2.40x10^{2}$	2.3802	±0.0013	$5.37x10^{3}$	3.7299	±0.6078
01/15/85	A	$6.83x10^{3}$	3.3802	±0.00	$1.40x10^{1}$	1.4924	±0.8792	$1.18x10^{2}$	2.0745	±0.2688
	S	$4.90x10^{2}$	2.7044	±0.4575	$5.60x10^{1}$	1.4343	±0.8308	$3.46x10^{2}$	2.5393	±0.2754

(Continued)

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[a] Aqueous Sample [b] Sediment Sample

[c] *Salmonella livingstone* counts/gram dry weight following spiking on 2/25/84 were $6.7x10^{5}$ (aqueous fraction) and $1.0x10^{6}$ (sediment fraction). No *Salmonella* was recovered on samples collected thereafter.

TABLE F-5 (Continued)

		Total Coliform /100 mL			Fecal Coliform /100 mL			Fecal Streptococci /100 mL		
		Geometric Mean	Log G.M.	Log S.D.	Geometric Mean	Log G.M.	Log S.D.	Geometric Mean	Log G.M.	Log S.D.
05/17/85	A	2.07×10^4	4.3152	±0.0122	8.4×10^1	1.9260	±0.2738	2.91×10^3	3.4634	±0.5413
	S	4.74×10^3	3.6755	±0.3598	5.0×10^1	1.6951	±0.5774	3.70×10^3	3.5686	±0.1631
09/11/85	A[a]	1.67×10^5	5.2231	±0.3122	1.7×10^3	3.2321	±0.1834	1.13×10^3	3.0512	±0.5618
	S[b]	1.32×10^4	4.1201	±0.2047	3.0	0.4771	±0.00	5.16×10^3	3.7123	±0.7732
02/18/86	A	3.75×10^2	2.5746	±0.6282	3.03	0.4811	±0.2565	5.56×10^1	1.7456	±0.2870
	S	6.68×10^3	3.8251	±0.4327	5.36	0.7295	±0.5220	1.97×10^4	4.2955	±0.1694

[a] Aqueous Sample
[b] Sediment Sample

APPENDIX G

VIRUS DATA

TABLE G-1. COMPILED VIRUS TITRATIONS FOR NEW ORLEANS LAGOON RAW DATA

Days	Geometric mean/S.D. PFU/100 mL wet weight	Geometric mean/S.D. PFU/gram dry weight	Thermal Control
0-1[a]	$7.4x10^5/\pm0.03$	$1.9x10^5\pm0.2$	$7.9x10^5$
0-2[b]	$8.1x10^5/\pm0.02$	$9x10^4\pm4.0$	
30-1	$1.7x10^3/\pm0.4$	$1.6x10^2\pm0.3$	$1.4x10^6$
30-2	$7.5x10^4/\pm0.1$	$1.3x10^4\pm0.3$	
60-1	<7.0[c]/±0.3	---[d]	$2.1x10^5$
60-2	$1.1x10^4/\pm0.1$	$3.3x10^3\pm1.1$	
90-1	<10.0/ ---	---	$6.0x10^4$
90-2	$6.4x10^3/\pm0.04$	$7.9x10^2$	
120-1	<10.0/ ---	---	$2.0x10^4$
120-2	$2.2x10^2/\pm0.1$	$3.0x10^1\pm0.8$	
180-1	<10.0/ ---		$6.0x10^3$
180-2	<10.0/ ---		

[a] Top sample
[b] Bottom sample
[c] Less than detection limits
[d] Not done

TABLE G-2. COMPILED VIRUS TITRATIONS FOR PORT ARANSAS LAGOON RAW DATA

Days	Geometric mean/S.D. PFU/100 mL wet weight	Geometric mean/S.D. PFU/gram dry weight	Thermal Control
0-1[a]	2.2x10^5/±0.2	1.7x10^4±0.2	8.0x10^5
0-2[b]	3.5x10^5/±0.2	3.6x10^4±1.5	
30-1	77.0x10^2/±0.6	3.1x10^1±1.2	1.4x10^6
30-2	9.9x10^2/±0.03	9x10^2±0.5	
60-1	11.0/±0.2	---[c]	9.0x10^4
60-2	6.4x10^2/±0.9	1.1x10^2±0.4	
90-1	9.0/±0.07	---	5.1x10^4
90-2	1.1x10^2/±0.2	7.0±0.6	
120-1	<10/ ---[d]	---	
120-2	9.0/±0.07	---	
180-1	<10/ ---	---	
180-2	<10/ ___	---	

[a] Top sample
[b] Bottom sample
[c] Not done
[d] Less than detection limits

TABLE G-3. POLIOVIRUS RECOVERY, NEW ORLEANS LAGOON

Sample	PFU/100 ML					
	Day 1	Day 30	Day 60	Day 90	Day 120	Day 180
Thermal Control[a]	7.9×10^5	1.4×10^6	2.1×10^5	6.0×10^4	2.0×10^4	6.0×10^3
1-1[b]	8.2×10^5	1.1×10^3	<10	<10	<10	<10
1-2[c]	7.9×10^5	5.5×10^4	7.5×10^3	5.6×10^3	1.9×10^2	<10
2-1	6.9×10^5	1.2×10^3	<10	<10	<10	<10
2-2	8.8×10^5	9.2×10^4	1.0×10^4	6.7×10^3	2.5×10^2	<10
3-1	7.9×10^5	9.8×10^2	<10	<10	<10	<10
3-2	8.1×10^5	8.9×10^4	1.3×10^4	6.5×10^3	1.7×10^2	<10
4-1	6.8×10^5	9.8×10^3	2	<10	<10	<10
4-2	7.6×10^5	7.0×10^4	1.2×10^4	7.1×10^3	2.3×10^2	<10
5-1	7.5×10^5	1.1×10^3	<10	<10	<10	<10
5-2	8.4×10^5	7.5×10^4	1.1×10^4	6.3×10^3	2.8×10^2	<10

[a] Vials of stock virus suspension placed in a sealed container and deposited in the sediment layer

[b] liquid phase

[c] sediment phase

TABLE G-4. POLIOVIRUS RECOVERY, PORT ARANSAS LAGOON

Sample	PFU/100 ML					
	Day 1	Day 30	Day 60	Day 90	Day 120	Day 180
Thermal Control[a]	8.0×10^{5}	1.4×10^{6}	9.0×10^{4}	5.1×10^{4}	2.3×10^{4}	5.9×10^{3}
1-1[b]	1.0×10^{5}	3.7×10^{2}	22	7	<10	<10
1-2[c]	3.6×10^{5}	9.8×10^{2}	1.3×10^{3}	1.4×10^{2}	7	<10
2-1	1.6×10^{5}	12	7	<10	<10	<10
2-2	4.9×10^{5}	9.7×10^{2}	1.5×10^{3}	1.6×10^{2}	<10	<10
3-1	3.2×10^{5}	5.3×10^{1}	<10	<10	<10	<10
3-2	4.4×10^{5}	8.9×10^{2}	1.5×10^{3}	1.3×10^{2}	8	<10
4-1	3.2×10^{5}	1.6×10^{2}	<10	<10	<10	<10
4-2	4.6×10^{5}	1.0×10^{3}	1.4×10^{3}	52	<10	<10
5-1	3.2×10^{5}	7.5×10^{1}	<10	<10	<10	<10
5-2	3.8×10^{5}	1.1×10^{3}	2.7×10^{3}	1.1×10^{2}	<10	<10

[a] Vials of stock virus suspension placed in a sealed container and deposited in the sediment layer

[b] liquid phase

[c] sediment phase

APPENDIX H

PARASITOLOGIC DATA

TABLE H-1. SUMMARY OF THE RESULTS OF THE PARASITOLOGIC EXAMINATION OF LAGOON-STORED SLUDGE FOR *ASCARIS* EGGS

Date of collection	Storage time in months	Mean no. of viable eggs/10gdw (±S.D.)[a]	Mean no. of total eggs/10gdw (±S.D.)[a]	Mean percent viability (±S.D.)
	New Orleans Lagoon			
02-06-84[b]	(24 hrs.)	194 (82)	2335 (813)	8.4% (1.6%)
05-09-84	3.0	398 (43)	2560 (456)	16.2% (1.2%)
08-06-84	6.0	134 (70)	1491 (704)	9.3% (1.8%)
11-14-84	9.3	64 (18)	1855 (556)	3.6% (1.0%)
02-11-85	12.2	2 (1.7)	718 (247)	0.3% (0.2%)
05-14-85	15.3	0	960 (210)	0.0%
08-12-85	18.2	0	871 (362)	0.0%
12-19-85	22.5	0	699 (239)	0.0%
03-01-86	24.7	0	412 (190)	0.0%
	Port Aransas Lagoon			
02-25-84	(24 hrs.)	64 (26)	1019 (318)	6.3% (1.9%)
06-04-84	3.4	21 (11)	1415 (523)	1.5% (0.5%)
09-10-84	6.5	0.2 (0.6)	896 (263)	0.02% (0.06%)
01-15-85	10.6	0	506 (179)	0.0%
05-17-85	14.7	0	605 (227)	0.0%
09-17-85	18.8	0	522 (271)	0.0%
02-18-86	23.7	0	264 (83)	0.0%
07-07-86	28.5	0	279 (141)	0.0%

[a] Mean number of eggs 110 gram dry weight; eggs examined after culturing.

[b] Samples on this date were analyzed using Procedure A and included both top and bottom layer sludge. All subsequent samples shown were analyzed using Procedure B and included only bottom layer sludge.

TABLE H-2. RESULTS OF THE PARASITOLOGIC EXAMINATION OF LAGOON-STORED SLUDGE FOR *ASCARIS* EGGS - NEW ORLEANS, LA.

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
02-06-84	0	Sludge prior to spiking	(0/100 mL)	(1/100 mL)	0.0%	-[b]	100 mL
		Eggs in sludge spike	(658/mL)	(4800/mL)	13.7%	-	SC[c]
	Top layer samples						
02-06-84[d]	0	11	249	3101	8.0%	4.1%	100 mL
		21	237	3717	6.4%	3.8%	100 mL
		31	200	2555	7.8%	5.4%	100 mL
		41	119	1740	6.8%	4.2%	100 mL
		51	187	2285	8.2%	3.4%	100 mL
		mean	198	2680	7.4%	4.2%	100 mL
		(S.D.)	(51)	(760)	(0.7%)	(0.7%)	(-)
05-14-85	15.3	31	0	3/50 gww	0.0%	0.02%	50 g
		41	0	3/80 gww	0.0%	0.08%	80 g

(Continued)

TABLE H-2 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
Bottom layer samples							
02-06-84[d]	0	12	204	2830	7.2%	3.7%	100 mL
		22	163	1856	8.8%	5.4%	100 mL
		22D[e]	180	2169	8.3%	-	100 mL
		32	388	3235	12.0%	4.0%	100 mL
		42	114	1079	10.6%	6.2%	100 mL
		52	91	1120	8.1%	5.6%	100 mL
		mean	190	2048	9.2%	5.0%	100 mL
		(S.D.)	(106)	(880)	(1.8%)	(1.1%)	(-)
02-11-85	12.2	12A	0	627	0.0%	8.0%	53.0 g
		12B	2	550	0.4%	8.7%	53.5 g
		22A	0	929	0.0%	10.0%	49.0 g
		22B	2	442	0.5%	11.3%	51.0 g
		32A	2	972	0.2%	8.8%	50.5 g
		32B	0	659	0.0%	10.3%	48.5 g
		42A	5	1178	0.4%	8.0%	52.5 g
		42B	2	370	0.5%	11.4%	48.5 g
		52A	4	885	0.5%	10.1%	49.0 g
		52B	2	563	0.4%	11.4%	48.5 g
		mean	2	718	0.3%	9.8%	50.4 g
		(S.D.)	(1.7)	(260)	(0.2%)	(1.4%)	(2.0 g)

(Continued)

TABLE H-2 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
05-14-85	15.3	12A	0	1093	0.0%	11.8%	50.0 g
		12B	0	775	0.0%	9.7%	50.0 g
		22A	0	834	0.0%	11.5%	51.0 g
		22B	0	1258	0.0%	8.6%	50.0 g
		32A	0	1288	0.0%	11.3%	52.0 g
		32B	0	938	0.0%	11.9%	50.0 g
		42A	0	686	0.0%	9.8%	50.0 g
		42B	0	1138	0.0%	9.9%	50.0 g
		52A	0	706	0.0%	8.9%	32.0 g
		52B	0	884	0.0%	13.1%	50.0 g
		mean	0	960	0.0%	10.7%	48.5 g
		(S.D.)	(-)	(222)	(-)	(1.5%)	(5.8 g)
08-12-85	18.2	12A	0	1174	0.0%	7.1%	50.0 g
		12B	0	1036	0.0%	8.5%	50.0 g
		22A	0	498	0.0%	9.2%	50.0 g
		22B	0	1439	0.0%	1.7%	50.0 g
		32A	0	653	0.0%	10.5%	50.0 g
		32B	0	215	0.0%	5.1%	50.0 g
		42A	0	640	0.0%	7.4%	50.0 g
		42B	0	1120	0.0%	7.1%	50.0 g
		52A	0	1214	0.0%	9.7%	50.0 g
		52B	0	725	0.0%	7.2%	50.0 g
		mean	0	871	0.0%	7.4%	50 g
		(S.D.)	(-)	(382)	(-)	(2.5%)	(-)

(Continued)

TABLE H-2 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
05-09-84	3.1	12A[f]	398	2799	14.2%	7.5%	100 mL
		12B[f]	372	2362	15.7%	9.1%	100 mL
		22A	449	2627	17.1%	9.1%	100 mL
		22B	431	2831	15.2%	9.6%	100 mL
		32A	389	2317	16.8%	8.2%	100 mL
		32B	333	1975	16.9%	7.0%	100 mL
		42A	350	2296	15.2%	7.1%	100 mL
		42B	375	2063	18.2%	11.3%	100 mL
		52A	414	2536	16.3%	8.1%	100 mL
		52B	469	2798	16.8%	8.1%	100 mL
		mean	398	2460	16.2%	8.5%	100 mL
		(S.D.)	(43)	(307)	(1.2%)	(1.3%)	(-)
08-06-84	6.0	12A	155	2363	6.6%	4.1%	52.0 g
		12B	79	677	11.7%	2.3%	52.0 g
		22A	64	701	9.1%	8.6%	52.0 g
		22B	103	1113	9.3%	8.5%	50.0 g
		32A	108	1311	8.2%	7.0%	51.0 g
		32B	122	1367	8.9%	6.6%	52.0 g
		42A	96	1318	7.3%	9.6%	52.0 g
		42B	193	2339	8.3%	5.1%	57.0 g
		52A	115	938	12.3%	6.5%	10.0 g
		52B	302	2783	10.8%	4.6%	55.0 g
		mean	134	1491	9.3%	6.3%	48.3 g
		(S.D.)	(70)	(743)	(1.8%)	(2.3%)	(13.6 g)

(Continued)

TABLE H-2 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
11-14-84	9.3	12A	53	1823	2.9%	6.5%	52.0 g
		12B	59	1500	3.9%	4.4%	50.0 g
		22A	61	1961	3.1%	9.2%	52.0 g
		22B	94	2061	4.6%	9.3%	49.0 g
		32A	38	1670	2.3%	11.2%	50.0 g
		32B	78	3282	2.4%	3.9%	55.0 g
		42A	73	1702	4.5%	10.4%	50.0 g
		42B	79	2074	3.8%	5.2%	51.0 g
		52A	36	1236	2.9%	8.4%	50.0 g
		52B	67	1236	5.4%	8.5%	51.0 g
		mean	64	1855	3.6%	7.7%	51.0 g
		(S.D.)	(18)	(586)	(1.0%)	(2.5%)	(1.7 g)
12-19-85	22.5	12A	0	935	0.0%	7.3%	50.0 g
		12B	0	885	0.0%	7.1%	50.0 g
		22A	0	690	0.0%	8.5%	50.0 g
		22B	0	412	0.0%	12.1%	50.0 g
		32A	0	868	0.0%	6.5%	50.0 g
		32B	0	980	0.0%	7.0%	50.0 g
		42A	0	312	0.0%	8.5%	50.0 g
		42B	0	929	0.0%	6.1%	50.0 g
		52A	0	519	0.0%	8.3%	50.0 g
		52B	0	463	0.0%	9.6%	50.0 g
		mean	0	699	0.0%	8.1%	50.0 g
		(S.D.)	(-)	(252)	(-)	(1.8%)	(-)

(Continued)

TABLE H-2 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
03-01-86	24.7	12A	0	407	0.0%	12.3%	50.0 g
		12B	0	221	0.0%	8.4%	50.0 g
		22A	0	287	0.0%	11.3%	50.0 g
		22B	0	174	0.0%	6.1%	50.0 g
		32A	0	262	0.0%	7.8%	50.0 g
		32B	0	443	0.0%	8.8%	50.0 g
		42A	0	489	0.0%	13.3%	50.0 g
		42B	0	539	0.0%	9.9%	50.0 g
		52A	0	432	0.0%	7.4%	50.0 g
		52B	0	867	0.0%	12.8%	50.0 g
		mean	0	412	0.0%	9.8%	50.0 g
		(S.D.)	(-)	(200)	(-)	(2.5%)	(-)

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[a] Percent solids estimated as: $\frac{\text{Weight of total solids}}{\text{100 mL sludge}} \times 100$

[b] Not done

[c] SC = Stoll Counts

[d] Acid/Alcohol - ether concentration method used in recovery procedure. Ether affected Ascaris egg viability and recovery.

[e] Duplicate sample

[f] A and B samples taken from same location in lagoon

TABLE H-3. RESULTS OF THE PARASITOLOGIC EXAMINATION OF LAGOON-STORED SLUDGE FOR ASCARIS EGGS - PORT ARANSAS, TEXAS

Collection Date	Storage time (months)	Sample no.		No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
02-25-84	0	Sludge prior to spike[b]		-[c]	-			-
		Eggs in sludge spike		(658/mL)	(4800/mL)	13.7%	-	SC[d]
Top layer sludge								
02-25-84[e]	0	11		47	667	7.0%	17.4%	100 mL
		21		46	724	6.4%	18.4%	100 mL
		31		93	1262	7.4%	17.2%	100 mL
		41		53	915	5.8%	19.6%	100 mL
		51		22	810	2.7%	19.0%	100 mL
			mean	52	876	5.9%	18.3%	100 mL
			(S.D.)	(26)	(235)	(1.9%)	(1.0%)	(-)

(Continued)

TABLE H-3 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
05-17-85	14.7	21	0	(2/80gwwt)	0	0.17%	80 g
		21D[f]	0	(1/80gwwt)	0	0.17%	80 g
		31	0	(1/80gwwt)	0	0.15%	80 g
		31D[f]	0	(0/80gwwt)	0	0.15%	80 g
09-17-85	18.8	11	0	(0/100gwwt)	0	0.21%	100 g
		31	0	(0/100gwwt)	0	1.19%	100 g
Bottom layer sludge							
02-25-84[e]	0	12	72	1307	5.5%	15.8%	100 mL
		22	42	1029	4.1%	13.7%	100 mL
		32	102	1102	9.3%	13.6	100 mL
		42	55	730	7.5%	8.0%	100 mL
		42D[f]	76	933	8.1%	-	100 mL
		52	98	1732	5.6%	7.8%	100 mL
		mean	74	1139	6.7%	11.8%	100 mL
		(S.D.)	(23)	(347)	(1.9%)	(3.6%)	(-)

(Continued)

TABLE H-3 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
06-04-84	3.4	12A[g]	25	1633	1.5%	14.1%	30.0 g
		12B[g]	17	707	2.4%	14.1%	30.0 g
		22A	13	1114	1.2%	15.8%	30.0 g
		22B	11	1023	1.1%	13.7%	100.0 mL
		32A	35	2298	1.5%	16.2%	30.0 g
		32B	18	1352	1.3%	14.5%	30.0 g
		42A	42	1777	2.4%	14.2%	30.0 g
		42B	23	1695	1.4%	14.2%	30.0 g
		52A	11	709	1.6%	15.6%	30.0 g
		52B	14	1838	0.8%	13.8%	30.0 g
		mean	21	1415	1.5%	14.6%	-[c]
		(S.D.)	(10)	(523)	(0.5%)	(0.9%)	-
09-10-84	6.5	12A	0	527	0.0%	15.9%	34.0 g
		12B	0	995	0.0%	15.5%	31.0 g
		22A	0	1054	0.0%	16.8%	30.0 g
		22B	0	986	0.0%	16.9%	32.0 g
		32A	2	988	0.2%	16.3%	31.0 g
		32B	0	594	0.0%	17.5%	33.0 g
		42A	0	1120	0.0%	17.6%	31.0 g
		42B	0	505	0.0%	17.7%	30.0 g
		52A	0	1267	0.0%	18.0%	30.0 g
		52B	0	925	0.0%	17.5%	31.0 g
		mean	0.2	896	0.02%	17.0%	31.3 g
		(S.D.)	(0.6)	(263)	(0.06%)	(0.8%)	(1.3 g)

(Continued)

TABLE H-3 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
01-15-85	10.6	12A	0	675	0.0%	10.6%	34.0 g
		12B	0	461	0.0%	10.0%	35.0 g
		22A	0	375	0.0%	15.5%	30.0 g
		22B	0	379	0.0%	13.9%	30.5 g
		32A	0	581	0.0%	13.8%	31.0 g
		32B	0	471	0.0%	14.5%	30.0 g
		42A	0	864	0.0%	16.2%	30.0 g
		42B	0	631	0.0%	13.4%	35.0 g
		52A	0	319	0.0%	13.9%	32.5 g
		52B	0	308	0.0%	14.5%	30.0 g
		mean	0	506	0.0%	13.6%	31.8 g
		(S.D.)	(-)	(179)	(-)	(1.9%)	(2.1 g)
05-17-85	14.7	12A	0	301	0.0%	6.2%	30.0 g
		12B	0	640	0.0%	2.5%	35.0 g
		22A	0	402	0.0%	7.6%	35.0 g
		22B	0	682	0.0%	5.2%	33.0 g
		32A	0	1058	0.0%	2.6%	32.0 g
		32B	0	744	0.0%	7.2%	31.0 g
		42A	0	312	0.0%	4.6%	30.0 g
		42B	0	656	0.0%	6.1%	34.0 g
		52A	0	556	0.0%	7.8%	33.0 g
		52B	0	694	0.0%	9.9%	39.0 g
		mean	0	605	0.0%	6.0%	33.2 g
		(S.D.)	(-)	(227)	(-)	(2.3%)	(2.7 g)

(Continued)

TABLE H-3 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
09-17-85	18.8	12A[h]	-	-	-	4.1%	32.0 g
		12B	0	440	0.0%	5.5%	30.0 g
		22A	0	649	0.0%	3.8%	30.0 g
		22B	0	193	0.0%	11.2%	30.0 g
		32A	0	361	0.0%	7.1%	34.0 g
		32B	0	430	0.0%	8.2%	31.0 g
		42A	0	1050	0.0%	2.4%	30.0 g
		42B	0	749	0.0%	1.4%	30.0 g
		52A	0	230	0.0%	9.9%	45.0 g
		52B	0	593	0.0%	6.8%	30.0 g
		mean	0	522	0.0%	6.0%	32.2 g
		(S.D.)	(-)	(271)	(-)	(3.2%)	(4.7 g)
02-18-86	23.7	12A	0	175	0.0%	6.3%	30.0 g
		12B	0	280	0.0%	6.3%	30.0 g
		22A	0	178	0.0%	8.8%	30.0 g
		22B	0	428	0.0%	8.8%	30.0 g
		32A	0	352	0.0%	1.8%	30.0 g
		32B	0	259	0.0%	1.8%	30.0 g
		42A	0	179	0.0%	8.0%	30.0 g
		42B	0	238	0.0%	8.0%	30.0 g
		52A	0	236	0.0%	7.5%	30.0 g
		52B	0	316	0.0%	7.5%	30.0 g
		mean	0	264	0.0%	6.5%	30.0 g
		(S.D.)	(-)	(83)	(-)	(2.6%)	(-)

(Continued)

TABLE H-3 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
07-07-86	28.3	12	0	180	0.0%	7.4%	30.0 g
		22	0	86	0.0%	7.0%	30.0 g
		32	0	404	0.0%	6.7%	30.0 g
		42	0	403	0.0%	7.8%	30.0 g
		52	0	324	0.0%	8.0%	30.0 g
		mean	0	279	0.0%	7.4%	30.0 g
		(S.D.)	(-)	(141)	(-)	(0.5%)	(-)

[a] Percent solids estimated as: $\frac{\text{Weight of total solids}}{\text{100 mL sludge}} \times 100$

[b] Sample not sent to laboratory
[c] Not done
[d] SC = Stoll Count
[e] Acid/Alcohol-Ether used in recovery procedure for top samples collected on 2-25-84.
[f] Duplicate sample
[g] A and B samples collected in same area of lagoon
[h] Sample lost due to breakage of container

TABLE H-4. RESULTS OF THE EXAMINATION OF CONTROL SAMPLES FROM NEW ORLEANS LAGOON FOR *ASCARIS* EGGS

Collection Date	Storage time (months)	No. viable eggs/10gdw[a]	Total no. eggs/10gdw	Percent viable	Percent solids[b]	Sample size (wet weight or volume)
Eggs in PVC Tube						
02-06-84[c]	0	(936/100mL)	(11300/100mL)	8.3%	-[e]	100 mL
05-09-84	3.1	19900[d]	120000	16.6%	1.9%	100 mL
08-06-84	6.0	1322	10200	13.0%	4.0%	50 g
11-14-84	9.3	7171	102300	7.0%	2.9%	50 g
02-11-85	12.2	0	63100	0.0%	0.8%	50 g
05-14-85	15.3	54	54600	0.1%	3.6%	50 g
05-23-85	15.6	92	80900	0.1%	3.4%	51 g
08-12-85	18.2	0	66800	0.0%	5.7%	32 g
12-19-85	22.5	0	49400	0.0%	0.6%	50 g
03-01-86	24.7	0	41900	0.0%	0.3%	50 g
Eggs in distilled water in vials						
02-06-84	0	(1028/20mL)	(1133/20mL)	90.7%	-[e]	20 mL
05-09-84	3.1	(981/20mL)	(1075/20mL)	91.3%	-	20 mL
08-06-84	6.0	(0/20mL)	(1168/20mL)	0.0%	-	20 mL
11-14-84	9.3	(0/20mL)	(1172/20mL)	0.0%	-	20 mL
02-11-85	12.2	(0/20mL)	(1478/20mL)	0.0%	-	20 mL
05-14-85	15.3	(0/20mL)	(906/20mL)	0.0%	-	20 mL
08-12-85[f]	-	-	-	-	-	-
12-19-85[f]	-	-	-	-	-	-
03-01-86[f]	-	-	-	-	-	-

[a] Number of eggs/10 gram dry weight. Eggs examined after culturing.

[b] Percent solids estimated as: $\frac{\text{Weight of total solids}}{\text{100 mL sludge}} \times 100$

[c] Acid/Alcohol-Ether concentration method used in recovery procedure on this date.

[d] Egg numbers greater than 10,000 are rounded off to nearest hundred.

[e] Not done.

[f] No vials remaining.

TABLE H-5. RESULTS OF THE EXAMINATION OF CONTROL SAMPLES FROM PORT ARANSAS LAGOON FOR ASCARIS EGGS

Collection Date	Storage time (months)	No. viable eggs/10gdw[a]	Total no. eggs/10gdw[a]	Percent viable	Percent solids[b]	Sample size (wet weight or volume)
Eggs in PVC Tube						
02-25-84[c]	0	(49/100mL)	(518/100mL)	9.5%	-[d]	100 mL
06-04-84	3.4	1	28	3.4%	4.0%	100 mL
08-06-84	5.4	0	99	0.0%	6.7%	53 g
09-10-84	6.5	0	77	0.0%	3.9%	55 g
01-15-85	10.6	0	49	0.0%	1.2%	33 g
05-17-85	14.7	0	25	0.0%	0.8%	50 g
09-17-85	18.8	0	71	0.0%	0.6%	50 g
02-18-86	23.7	0	0	-	0.7%	50 g
07-07-86	28.3	0	25	0.0%	11.8%	30 g
Eggs in distilled water in vials						
02-25-84	0	(1274/20mL)	(1375/20mL)	92.7%	-[d]	20 mL
06-04-84	3.4	(278/20mL)	(1033/20mL)	26.9%	-	20 mL
09-10-84	6.5	(0/20mL)	(1570/20mL)	0.0%	-	20 mL
01-15-85	10.6	(0/20mL)	(1226/20mL)	0.0%	-	20 mL
05-17-85	14.7	(0/20mL)	(814/20mL)	0.0%	-	20 mL
09-17-85	18.8	(0/20mL)	(1336/20mL)	0.0%	-	20 mL
02-18-86	23.7	(0/20mL)	(513/20mL)	0.0%	-	20 mL
07-07-86	28.3	(0/20mL)	(326/20mL)	0.0%	-	20 mL

[a] Number of eggs/10 gram dry weight. Eggs examined after culturing.

[b] Percent solids estimated as: $\frac{\text{Weight of total solids}}{\text{100 mL sludge}} \times 100$

[c] Acid/Alcohol-Ether concentration method used in recovery procedure on this date.

[d] Not done.

TABLE H-6. SUMMARY OF RESULTS OF THE PARASITOLOGIC EXAMINATION FOR ASCARIS EGGS OF NEW ORLEANS SLUDGE STORED IN LARGE PLASTIC COLUMN

Collection Date	Storage time (months)	Type of Sample	Mean no. viable eggs/10gdw (± S.D.)[a]	Mean total no. eggs/10gdw (± S.D.)[a]	Mean Percent Viable
07-15-85	0	New Orleans sludge prior to spike	0	0	
		Eggs in sludge spike	(3107/10mL)	(3430/10mL)	90.6%
07-16-85[b]	0	Top layer sludge	758 (189)	830 (227)	91.9% (2.6%)
08-16-85	1.0	" "	0	0	0.0
07-16-86	12.0	" "	0	0	0.0
07-16-85[b]	0	Bottom layer sludge	1643 (396)	1813 (459)	90.8% (1.0%)
08-16-85	1.0	" "	9087 (287)	9995 (303)	90.9% (0.1%)
10-25-85	3.3	" "	10625 (590)	11493 (573)	92.5% (0.5%)
01-27-86	6.4	" "	8845(4303)	9898(4963)	90.1% (2.3%)
04-16-86	9.0	" "	4171 (96)	5152 (181)	81.0% (1.0%)
07-16-86	12.0	" "	240 (33)	1393 (678)	21.4%(13.9%)
10-20-86	15.2	" "	0	9006 (800)	0.0
1-29-87	18.5	" "	0	9021(1278)	0.0

[a] Mean number of eggs/10 gram dry weight. Eggs examined after culturing.
[b] Samples taken immediately after spike added and mixed with sludge.

TABLE H-7. RESULTS OF THE PARASITOLOGIC EXAMINATION FOR ASCARIS EGGS IN NEW ORLEANS SLUDGE STORED IN LARGE PLASTIC COLUMN

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
07-15-85	0	New Orleans sludge prior to spike	0	0		9.7%	40 g
		Eggs in sludge spike	(3107/10mL)	(3430/10mL)	90.6%	-[b]	30 mL
	Top layer sludge						
07-16-85[c]	0	A[d]	924	1031	89.6%	9.7%	50 g
		B[d]	798	874	91.3%	9.7%	50 g
		C[d]	553	584	94.7%	9.7%	50 g
		mean	758	830	91.9%	9.7%	50 g
		(S.D.)	(189)	(227)	(2.6%)	(-)	(-)
08-16-85	1.0	A	0	0		-	52 g
		B	0	0		-	51 g
07-16-86	12.0	A	0	0		0.09%	100 g
	Bottom layer sludge						
07-16-85[c]	0	A	1436	1568	91.6%	9.7%	50 g
		B	2099	2342	89.6%	9.7%	50 g
		C	1393	1529	91.1%	9.7%	50 g
		mean	1643	1813	90.8%	9.7%	50 g
		(S.D.)	(396)	(459)	(1.0%)	(-)	(-)

(Continued)

TABLE H-7 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
08-16-85	1.0	A	9290	10209	91.0%	10.0%	52 g
		B	8884	9781	90.8%	10.0%	50 g
		mean	9087	9995	90.9%	10.0%	51 g
		(S.D.)	(287)	(303)	(0.1%)		(1 g)
10-25-85	3.3	A	10208	11088	92.1%	7.2%	30 g
		B	11042	11898	92.8%	7.2%	30 g
		mean	10625	11493	92.5%	7.2%	30 g
		(S.D.)	(590)	(573)	(0.5%)	(-)	
01-27-86	6.4	A	12675	14298	88.6%	7.6%	30 g
		B	9671	10877	88.9%	7.6%	30 g
		C	4189	4518	92.7%	7.6%	30 g
		mean	8845	9898	90.1%	7.6%	30 g
		(S.D.)	(4303)	(4963)	(2.3%)	(-)	(-)
04-16-86	9.0	A	4239	5280	80.3%	4.2%	30 g
		B	4103	5024	81.7%	4.2%	30 g
		mean	4171	5152	81.0%	4.2%	30 g
		(S.D.)	(96)	(181)	(1.0%)	(-)	(-)
07-16-86	12.0	A	259	692	37.4%	0.7%	50 g
		B	259	2046	12.7%	0.7%	50 g
		C	202	1441	14.1%	0.7%	50 g
		mean	240	1393	21.4%	0.7%	50 g
		(S.D.)	(33)	(678)	(13.9%)	(-)	

(Continued)

TABLE H-7 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
10-20-86	15.2	A	0	8440	0.0%	3.7%	50 g
		B	0	9571	0.0	3.7%	50 g
		mean	0	9006	0.0%	3.7%	50 g
		(S.D.)	(-)	(800)	(-)	(-)	(-)
01-29-87	18.5	A	0	9290	0.0%	4.9%	50 g
		B	0	10143	0.0	4.9%	50 g
		C	0	7629	0.0%	4.9%	50 g
		mean	0	9021	0.0%	4.9%	50 g
		(S.D.)	(-)	(1278)	(-)	(-)	(-)

[a] Percent solids estimated as: $\frac{\text{Weight of total solids}}{\text{100 g sludge}} \times 100$

[b] Not done

[c] Samples taken immediately after spike added and mixed with sludge

[d] A, B, and C are subsample

TABLE H-8. SUMMARY OF RESULTS OF THE PARASITOLOGIC EXAMINATION FOR ASCARIS EGGS OF PORT ARANSAS SLUDGE STORED IN LARGE PLASTIC COLUMN

Collection Date	Storage time (months)	Type of Sample	Mean no. viable eggs/10gdw (± S.D.)[a]	Mean total no. eggs/10gdw (± S.D.)[a]	Mean Percent Viable (± S.D.)[a]
07-15-85	0	Port Aransas sludge prior to spike	0	0	
		Eggs in sludge spike	(3107/10mL)	(3430/10mL)	90.6%
07-16-85[b]	0	Top layer sludge	5599 (1371)	6193 (1521)	90.4% (0.5%)
08-16-85	1.0	" "	0	(4/105gwwt)	0.0
07-16-86	12.0	" "	0	0	0.0
01-29-87	18.5	" "	0	0	0.0
07-16-83[b]	0	Bottom layer sludge	327 (35)	376 (29)	86.7% (2.8%)
08-16-85	1.0	" "	924 (365)	1158 (407)	80.9% (3.1%)
10-25-85	3.3	" "	1064 (231)	1571 (231)	67.4% (4.8%)
01-27-86	6.4	" "	619 (244)	1526 (250)	40.3%(11.8%)
04-16-86	9.0	" "	49 (4)	603 (216)	8.9% (3.9%)
07-16-86	12.0	" "	0.7 (1.2)	1141 (34)	0.06% (0.1%)
10-20-86	15.2	" "	2 (3)	922 (27)	0.22%(0.22%)
01-29-87	18.5	" "	0	789 (380)	0.0

[a] Mean number of eggs/10 gram dry weight. Eggs examined after culturing.
[b] Samples taken immediately after spike added and mixed with sludge.

TABLE H-9. RESULTS OF THE PARASITOLOGIC EXAMINATION FOR *ASCARIS* EGGS IN PORT ARANSAS SLUDGE STORED IN LARGE PLASTIC COLUMN

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
07-15-85	0	Port Aransas sludge prior to spike	0	0		9.7%	49 g
		Eggs in sludge spike	(3107/10mL)	(3430/10mL)	90.6%	-[b]	30 mL
	Top layer sludge						
07-16-85[c]	0	A[d]	4047	4480	90.3%	9.7%	50 g
		B[d]	6643	7384	90.0%	9.7%	50 g
		C[d]	6107	6715	90.9%	9.7%	50 g
		mean	5599	6193	90.4%	9.7%	50 g
		(S.D.)	(1371)	(1521)	(0.5%)	(-)	(-)
08-16-85	1.0	A	0	(3/55gwwt)	0	-	55 g
		B	0	(1/50gwwt)	0	-	50 g
07-16-86	12.0	A	0	0	-	0.25%	100 g
01-29-87	18.5	A	0	0	-	-	100 g

(Continued)

TABLE H-9 (continued)

Collection Date	Storage time (months)	Sample no.		No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
	Bottom layer sludge							
07-16-85[c]	0	A		295	348	84.6%	9.7%	50 g
		B		365	406	89.9%	9.7%	50 g
		C		321	375	85.6%	9.7%	50 g
			mean	327	376	86.7%	9.7%	50 g
			(S.D.)	(35)	(29)	(2.8%)	(-)	(-)
08-16-85	1.0	A		685	870	78.7%	18.4%	50 g
		B		1201	1446	83.1%	18.4%	50 g
			mean	924	1158	80.9%	18.4%	50 g
			(S.D.)	(365)	(407)	(3.1%)	(-)	(-)
10-25-85	3.3	A		1227	1734	70.8%	14.8%	30 g
		B		901	1408	64.0%	14.8%	30 g
			mean	1064	1571	67.4%	14.8%	30 g
			(S.D.)	(231)	(231)	(4.8%)	(-)	(-)
01-27-86	6.4	A		460	1609	28.6%	17.4%	30 g
		B		498	1245	40.0%	17.4%	30 g
		C		900	1724	52.2%	17.4%	30 g
			mean	619	1526	40.3%	17.4%	30 g
			(S.D.)	(244)	(250)	(11.8%)	(-)	(-)
04-16-86	9.0	A		52	450	11.6%	18.8%	30 g
		B		46	755	6.1%	18.8%	30 g
			mean	49	603	8.9%	18.8%	30 g
			(S.D.)	(4)	(216)	(3.9%)	(-)	(-)

(Continued)

TABLE H-9 (continued)

Collection Date	Storage time (months)	Sample no.	No. viable eggs/10gdw	Total no. eggs/10gdw	Percent viable	Percent[a] solids	Sample size (wet weight or volume)
07-16-86	12.0	A	0	1169	0.00%	18.6%	30 g
		B	2	1151	0.17%	18.6%	30 g
		C	0	1103	0.00%	18.6%	30 g
		mean	0.7	1141	0.06%	18.6%	30 g
		(S.D.)	(1.2)	(34)	(0.10%)		(-)
10-20-86	15.2	A	4	941	0.43%	18.6%	30 g
		B	0	903	0.00%	18.6%	30 g
		mean	2	922	0.22%	18.6%	30 g
		(S.D.)	(3)	(27)	(0.22%)	(-)	(-)
01-29-87	18.5	A	0	546	0.0%	15.5%	30 g
		B	0	594	0.0%	15.5%	30 g
		C	0	1226	0.0%	15.5%	30 g
		mean	0	789	0.0%	15.5%	30 g
		(S.D.)	(-)	(380)	(-)	(-)	(-)

[a] Percent solids estimated as: $\frac{\text{Weight of total solids}}{\text{100 g sludge}} \times 100$

[b] Not done

[c] Samples taken immediately after spike added and mixed with sludge

[d] A, B, and C are subsamples

TABLE H-10. RESULTS OF THE PARASITOLOGIC EXAMINATION FOR ASCARIS EGGS IN DISTILLED WATER IN VIALS STORED IN LARGE PLASTIC COLUMNS[a]

Collection Date	Time (months)	No. viable eggs[b]	Total no. eggs[b]	Percent Viable
	Vials stored in New Orleans column			
09-24-85	2.3	906	1286	70.5%
10-25-85	3.3	518	1806	28.7%
01-27-86	6.4	189	705	26.8%
04-16-86	9.0	270	409	66.0%
07-16-86	12.0	8	213	3.8%
10-20-86	15.2	0	669	0.0%
01-29-87	18.5	0	570	0.0%
	Vials stored in Port Aransas column			
09-24-85	2.3	1067	1220	87.5%
10-25-85	3.3	350	603	58.0%
01-27-86	6.4	2644	2908	90.9%
04-16-86	9.0	1694	2329	72.7%
07-16-86	12.0	7	363	1.9%
10-20-86	15.2	0	1254	0.0%
01-29-87	18.5	0	319	0.0%

[a] *Ascaris* eggs placed in sterile distilled water in 5 mL culture tubes and placed in a plastic column (1 ft. x 10 ft.) filled with either New Orleans sludge or Port Aransas sludge.

[b] Eggs examined after culturing.

TABLE H-11. QUALITY CONTROL DATA FOR PARASITOLOGIC ANALYSIS OF LAGOON-STORED SLUDGE FOR ASCARIS EGGS. A. PRECISION

Date Collected	Location	Sample no.	Total Eggs/100 gwwt Sample 1	 Sample 2	Mean	Standard Deviation	S.D. % of mean
02-06-84[a]	New Orleans	22	(1000/100mL)	(1169/100mL)	1085	120	11.1%
	"	31	(980/100mL)	(1069/100mL)	1025	63	6.2%
02-25-84[a]	Pt. Aransas	42	(649/100mL)	(750/100mL)	700	71	10.1%
05-07-84	New Orleans	52A	(2048/100mL)	(1577/100mL)	1813	333	18.4%
06-04-84	Pt. Aransas	22B	(1756/100mL)	(1401/100mL)	1579	251	15.9%
08-06-84	New Orleans	52B	691	272	482	296	61.4%
09-10-84	Pt. Aransas	12B	478	776	627	211	33.7%
11-14-84	New Orleans	42B	1030	879	955	107	11.2%
	"	52A	332	452	392	85	21.7%
01-15-85	Pt. Aransas	22A	580	482	531	69	13.0%
02-11-85	New Orleans	32A	794	857	826	45	5.4%
05-14-85	"	42A	672	620	646	37	5.7%
05-17-85	Pt. Aransas	52A	433	509	471	54	11.5%
07-16-85	New Orleans	N.O.C.2[b]	1524	1486	1505	27	1.8%
	"	P.A.C.1[c]	6520	4350	5435	1534	28.2%
	"	P.A.C.2[d]	394	364	379	21	5.5%
08-12-85	New Orleans	32A	686	548	617	98	15.9%
09-17-85	Pt. Aransas	52B	373	400	387	19	4.9%
10-25-85	"	N.O.C.2[b]	7983	8567	8275	413	5.0%
	"	P.A.C.2[d]	2567	2083	2325	342	14.7%
12-19-85	"	12B	508	630	569	86	15.1%
01-27-86	"	N.O.C.2[b]	10867	8266	9567	1839	19.2%
	"	N.O.C.2[b]	2800	2167	2484	448	18.0%
02-18-86	Pt. Aransas	52B	177	237	207	42	20.3%
03-01-86	New Orleans	12B	108	186	147	55	37.4%

(Continued)

TABLE H-11 (continued)

Date Collected	Location	Sample no.	Total Eggs/100 gwwt Sample 1	Sample 2	Mean	Standard Deviation	S.D. % of mean
04-16-86	"	N.O.C.2	2197	2090	2144	76	3.5%
	"	P.A.C.2	843	1417	1130	406	35.9%
07-07-86	Pt. Aransas	32	250	270	260	14	5.4%
07-16-86	New Orleans	P.A.C.2	2170	2136	2153	24	1.1%
10-20-86	"	N.O.C.2	3106	3522	3314	294	8.9%
	"	P.A.C.2	1750	1680	1715	49	2.9%
01-29-87	"	N.O.C.2	4518	3738	4128	552	13.4%
	"	P.A.C.2	847	920	884	52	5.9%

[a] Samples analyzed using Acid/Alcohol-Ether concentration method (Procedure A).

[b] N.O.C.2 = New Orleans sludge stored in large PVC column, bottom layer sample.

[c] P.A.C.1 = Pt. Aransas sludge stored in large PVC column, top layer sample.

[d] P.A.C.2 = Pt. Aransas sludge stored in large PVC column, bottom layer sample.

TABLE H-12. QUALITY CONTROL DATA FOR PARASITOLOGIC ANALYSIS OF LAGOON-STORED SLUDGE FOR ASCARIS EGGS. B. ACCURACY

Date	Location	Sample no.	Mean No. total eggs/sample[a]	No. eggs in spike	No. eggs recovered from spiked sample	Percent recovery[b]
02-06-84[c]	New Orleans	22	1085	1000	1588	50.3%
05-07-84	"	52A	1813	1000	2769	95.6%
06-04-84	Pt. Aransas	22B	1579	1000	2213	63.4%
09-10-84	"	12B	723	1000±17	1266	54.3%
11-14-84	New Orleans	42B	955	1000±17	1752	79.7%
	"	52A	392	1000±17	1110	71.8%
01-15-85	Pt. Aransas	22A	165	913±48	914	82.0%
02-11-85	New Orleans	32A	413	1000±51	1529	111.6%
05-14-85	"	42A	323	941±72	946	66.2%
05-17-85	Pt. Aransas	52A	141	633±13	460	50.4%
07-16-85	New Orleans	N.O.C.2[d]	753	1028±28	2134	134.3%
	"	P.A.C.2[e]	2718	1028±28	4015	126.2%
08-12-85	New Orleans	32A	309	1028±28	1277	94.2%
09-17-85	Pt. Aransas	52B	116	1028±28	815	68.0%
10-25-85	New Orleans	N.O.C.2[d]	2483	1028±28	3225	72.2%
	"	P.A.C.2[e]	698	1028±28	1320	60.5%
12-19-85	New Orleans	12B	285	1028±28	1078	77.1%
01-27-86	"	N.O.C.2[d]	2557	1017±28	2940	37.7%
	"	P.A.C.2[e]	797	1017±28	1305	50.0%
02-18-86	Pt. Aransas	52B	62	1017±28	788	71.4%
03-01-86	New Orleans	12B	74	1017±28	1014	92.4%
04-16-86	"	N.O.C.2[d]	643	1017±28	1393	73.7%
	"	P.A.C.2[e]	339	1017±28	970	62.0%
07-07-86	Pt. Aransas	32	78	1017±28	593	50.6%
07-16-86	New Orleans	P.A.C.2[e]	646	1017±28	1361	70.3%

(Continued)

TABLE H-12 (continued)

Date	Location	Sample no.	Mean No. total eggs/sample[a]	No. eggs in spike	No. eggs recovered from spiked sample	Percent recovery[b]
10-20-86	"	N.O.C.2[d]	1657	1017±28	2950	127.1%
	"	P.A.C.2[e]	515	1017±28	1346	81.7%
01-29-87	"	N.O.C.2	2064	1017±28	2529	45.7%
	"	P.A.C.2	265	1017±28	978	70.1%

[a] Mean number of Ascaris eggs in two subsamples, adjusted to correspond with wet weight of spiked sample

[b] Percent Recovery =

$$\frac{\text{(Total no. eggs recovered from spiked sample - mean no. eggs in samples)}}{\text{No. of eggs in spike}} \times 100$$

[c] Samples analyzed using Acid/Alcohol Ether concentration method. (Procedure A)

[d] N.O.C.2 = New Orleans sludge stored in large PVC column, bottom layer sample

[e] P.A.C.2 = Pt. Aransas sludge stored in large PVC column, bottom layer sample

TABLE H-13. SUMMARY OF INFECTIVITY OF CULTURED ASCARIS EGGS FOR MICE

Collection Date	Storage time (months)	Type of sample	No. of mice infected	Mean no. viable larvae infected/mouse	Mean % recovery of larvae/mouse	Mean recovery vs. control[a]
New Orleans Lagoon						
02-06-84	0	Sludge in lagoon	3	267	7.5%	52.4%
05-07-84	3.0	"	5	335	2.7%	37.0%
08-06-84	6.0	"	2	184	3.3.%	41.3%
11-14-84	9.3	"	2	124	1.6%	17.8%
02-11-85	12.2	"	1	9	0.0%	0.0%
05-14-85[b]	15.3	"	-[c]	-	-	-
02-06-84	0.0	Sludge in control tube	3	312	13.1%	50.4%
05-07-84	3.0	"	3	300	0.0%	0.0%
08-06-84	6.0	"	1	53	1.9%	23.8%
11-14-84	9.3	"	2	320	0.9%	10.0%
02-11-85[b]	12.2	"	-	-	-	-
02-06-84	0.0	Eggs in distilled water	3	346	4.3%	30.1%
05-07-84	3.0	"	3	327	30.3%	415.1%
08-06-84[b]	6.0	"	-	-	-	-

(Continued)

TABLE H-13 (continued)

Collection Date	Storage time (months)	Type of sample	No. of mice infected	Mean no. viable larvae infected/mouse	Mean % recovery of larvae/mouse	Mean recovery vs. control[a]
	Pt. Aransas Lagoon					
02-25-84	0.0	Sludge in lagoon	3	308	0.3%	1.1%
06-04-84	3.4	"	1	85	0.0%	0.0%
09-10-84[b]	6.5	"	-	-	-	-
02-25-84	0.0	Sludge in control tube	1	49	4.1%	15.4%
06-04-84[b]	3.4	"	-	-	-	-
02-25-84	0.0	Eggs in distilled water	4	318	20.8%	78.2%
06-04-84	3.4	"	1	278	6.5%	81.3%
09-10-84[b]	6.5	"	-	-	-	-
	Large plexiglass columns					
07-16-85	0.0	N.O.C.2[d]	-	-	-	-
08-16-85	1.0	"	-	-	-	-
10-25-85	3.3	"	4	300	19.3%	60.1%
01-27-86	6.4	"	4	299	26.8%	241.4%
04-16-86	9.0	"	4	260	3.5%	29.4%
07-16-86	12.0	"	1	25	0.0%	0.0%
10-20-86[b]	15.2	"	-	-	-	-
07-16-85	0.0	Eggs in dH_2O (N.O.C.2)	-	-	-	-
08-16-85	1.0	"	-	-	-	-

(Continued)

TABLE H-13 (continued)

Collection Date	Storage time (months)	Type of sample	No. of mice infected	Mean no. viable larvae infected/mouse	Mean % recovery of larvae/mouse	Mean recovery vs. control[a]
10-25-85	3.3	"	2	259	20.1%	86.6%
01-27-86	6.4	"	1	189	7.0%	63.1%
04-16-86	9.0	"	2	135	11.5%	96.6%
07-20-86	12.0	"	1	8	0.0%	0.0%
10-20-86[b]	15.2	"	-	-	-	-
07-16-85	0.0	P.A.C.2[e]	-	-	-	-
08-16-85	1.0	"	-	-	-	-
10-25-85	3.3	"	3	315	1.0%	3.1%
01-27-86	6.4	"	2	170	10.0%	90.1%
04-16-86	9.0	"	1	55	18.2%	150.3%
07-16-86[b]	12.0	"	-	-	-	-
07-16-85	0.0	Eggs in dH$_2$O (P.A.C.2)	-	-	-	-
08-16-85	1.0	"	-	-	-	-
10-25-85	3.3	"	2	175	19.4%	83.6%
01-27-86	6.4	"	4	300	13.3%	119.8%
04-16-86	9.0	"	3	116	11.2%	94.1%
07-16-86	12.0	"	1	7	0.0%	0.0%
10-20-86[b]	15.2	"	-	-	-	-

[a] Mean Percent Recovery of larvae from mice infected with eggs from sludge samples as compared to Mean Percent Recovery of larvae from mice infected with control eggs.

[b] At this date and thereafter, there were insufficient number of viable larvae for infecting a mouse.

[c] Not done

[d] N.O.C.2 = New Orleans large column, bottom layer sludge

[e] P.A.C.2 = Port Aransas large column, bottom layer sludge

TABLE H-14. DISTRIBUTION OF *ASCARIS* EGGS IN A COLUMN OF ANAEROBICALLY DIGESTED SLUDGE AFTER VARIOUS SETTLING TIMES

Depth of Sample	Time of Sample Collection								
	0 hrs	3 hrs	6 hrs	12 hrs	24 hrs	1 wk	1 mo	2 mo	3 mo
1 foot	75 ±24[a]	71 ±19	57 ±13	13 ± 5	3 ± 3	1 ± 1	0	0	0
2 feet	75 ±24	71 ±18	98 ±29	74 ±19	23 ±10	2 ± 1	0	0	0
3 feet	75 ±24	76 ±39	103 ±30	75 ±26	57 ±27	1 ± 1	0	0	0
4 feet	75 ±24	78 ±24	103 ±16	77 ±19	58 ±12	180 ±34	130 ±30	43 ±20	13 ± 4
5 feet	75 ±24	81 ±19	80 ±22	78 ±14	109 ±23	208 ±72	208 ±54	127 ±54	140 ±28
6 feet	75 ±24	97 ±21	104 ±31	88 ±24	159 ±46	205 ±53	187 ±48	172 ±68	113 ±31

[a] Mean number of *Ascaris* eggs/mL ± standard deviation.

TABLE H-15. PERCENTAGE OF ASCARIS EGGS AT DIFFERENT DEPTHS IN A COLUMN OF ANAEROBICALLY DIGESTED SLUDGE AFTER VARIOUS SETTLING TIMES

Depth of Sample (feet)	Time of Sample Collection								
	0 hrs	3 hrs	6 hrs	12 hrs	24 hrs	1 wk	1 mo	2 mo	3 mo
1	16.7[a]	15.0	10.5	3.2	0.7	0.2	0.0	0.0	0.0
2	16.6	15.0	18.0	18.3	5.6	0.3	0.0	0.0	0.0
3	16.7	16.0	18.9	18.5	13.9	0.2	0.0	0.0	0.0
4	16.7	16.5	18.9	19.0	14.2	30.2	24.8	12.6	4.9
5	16.6	17.1	14.7	19.3	26.7	34.8	39.6	37.1	52.6
6	16.7	20.4	19.0	21.9	38.9	34.3	35.6	50.3	42.5

[a] Percentage of all eggs collected at that time of sample collection

APPENDIX I

DEFINITIONS OF ACRONYMS

ASTM	American Society of Testing Materials
BGM	Green Monkey
CFU	Colony Forming Unit
COD	Chemical Oxygen Demand
CRF	Code of Federal Regulations
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
GC/MS	Gas Chromatography/Mass Spectrometry
gdw	gram x dry weight
LOG	Logarithm
MPN	Most Probable Number
N. O.	New Orleans (lagoon)
ND	Not detectable
NS	Not Significant
ORP	Oxidation Reduction Potential
P. A.	Port Aransas (lagoon)
PFRP	Process to Further Reduce Pathogens
PFU	Plaque Forming Unit
Polio 1	Poliovirus type 1
POWT	Publicly Owned Work Treatment
PSRP	Process to Significantly Reduce Pathogens
SD	Standard Deviation

SM	Standard Method for the Examination of Water and Wastes
Sp	Species
SU	Standard Units
TCLP	Toxicity Characteristics Leaching Procedure
TKN	Total Kjedahl Nihojes
TSS	Total Suspended Solid
YSI	Yellow Spring Instruments